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SKIN POTENTIAL AND IMPEDANCE RESPONSES WITH RECURRING SHOCK STIMULATION

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A slow electrical response following stimulation of the organism has been known, since Vigouroux in 1879 called attention to a direct current resistance change and Tarchanoff in 1890 reported an endosomatic "action current" response. Over 600 papers have been published to date on this "psychogalvanic phenomenon."

Electrodes have usually been attached to palmar and dorsal surfaces of the hand in humans and ear to footpad in animals. In cats the resistance level rises after sympathectomy and the resistance response cannot be elicited (Richter, 1929; Richter and Shaw, 1930; Schwartz, 1934). Evidence has been advanced for a possible parasympathetic factor in palmar resistance in humans and monkeys (Richter, 1929a), and polyphasic endosomatic potential waveform from palm-dorsum leads has been held to indicate sympathetic-parasympathetic balance (Odegaard, 1930).

On the other hand, polyphasic waveforms have been reported from *single* reacting skin areas (Darrow, 1929; Forbes, 1934), and Gildemeister (1928) has formulated an explanation of polyphasic waveform of the response in terms of a unitary neuro-glandular mechanism in which one potential represents an increase in permeability of sudorific cell membranes and the following one results from an exudation of positive ions from the cell interior through pores in the membranes.

There is evidence for more than one factor in phase angle measurements from resting skin. Strohl (1930) on the basis of conductivity data on unstimulated skin has suggested that two tissue structures with different phase angle characteristics are necessary to explain reactance-resistance curves for various frequencies. Cole (1932) has pointed out the entrance of a second factor at high frequencies in measurements of phase angle by himself and others on frog skin.

The present results indicate two *response* mechanisms in a given skin area which may show functional independence.

METHOD. *Electrodes.* For endosomatic potential response records electrodes consisted of $1 \times 1\frac{1}{2}$ inch zinc plates with gauze pads soaked in $\frac{1}{10}$ saturated zinc sulphate solution, attached to the palm and the dorsum of the left hand of the subject, and to the arm just above the elbow. The skin at the arm electrode was pierced to eliminate responses from this indifferent area and to effect a connection to the body fluids on the inner surface of the area investigated. An insulated hypodermic needle thrust through the skin of the arm and a wick electrode in the mouth were also used for the same purpose. Electrodes for audiofrequency impedance measurements were 1 cm.^{2} zinc, zinc sulfate on palm and dorsum and insulated needle through the skin of the arm. A larger zinc plate electrode (6 cm. diam.) was also used as the inactive electrode for comparison.

Recording. A Cambridge string galvanometer was used for the majority of the records.

To obtain a practically *pure potential* record a high resistance input vacuum tube amplifier was employed. It could be used either as a single stage balanced D.C. amplifier or as a Matthews (1934) balanced input to a second stage. The effective input resistance of this amplifier was approximately 6 megohms and resistance variations of 100,000 ohms produced no apparent deflection. The balanced pure resistance connection eliminated distortion from the use of condensers and from any applied voltage on the skin from the grid bias. The output from this balanced stage was observed direct on the string galvanometer for accurate recording of waveform but was coupled through 25 microfarad electrolytic condensers to a single stage with a 60 microampere Weston type 600 meter in a balanced output circuit for routine recording. A light aluminum rod pointer attached to this meter recorded in the light beam of the string galvanometer.

This meter recorder showed an initial lag of approximately 0.04 second due to the inertia of the meter, and approximately 0.25 second in reaching a 1 millivolt change of level. However, the recorder did not distort the waveform appreciably as shown when it was used simultaneously and in parallel with the string galvanometer.

Method for A.C. analysis. In order to obtain a further electrical analysis of the response our audiofrequency bridge recording was used. In this method an alternating current (approximately 40 μA) is passed through the skin which forms one arm of an alternating current Wheatstone bridge. The Wheatstone bridge gives, in usual fashion, the capacitance and resistance of the skin before and after the response. Our bridge is so designed, however, that the unbalance occurring *during* the galvanic skin response is recorded photographically from the figure on a cathode ray tube. This arrangement allows the registration of changes which occur too rapidly to be accurately followed by manual balancing of the bridge.

The amount of the *unbalance* is applied across one pair of deflecting plates of the cathode ray tube while a comparison voltage from a 100 ohm pure resistor (R_7, fig. 1) is applied across the other pair of plates. In this fashion the engineering technique of measuring phase angle changes on the cathode ray tube[1] by recording current versus voltage is applied to the bridge, allowing higher amplification and greater sensitivity than could be obtained by the use of the method without the bridge.

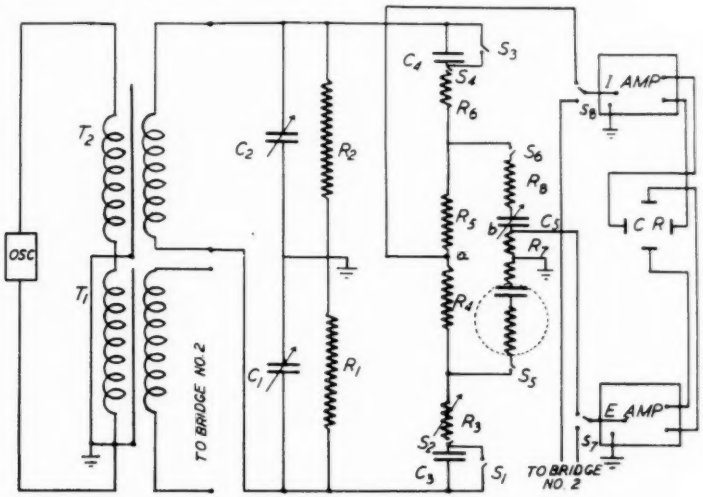

Fig. 1. Audiofrequency bridge and cathode ray circuit. *T1* and *T2* = shielded bridge transformer G. R. Co. type 578A; *C1*, *C2*, *R1*, *R2* = Wagner ground arrangement; *C3*, *C4* = blocking condensers 0.1 μF mica and 1000 $\mu\mu$F variable air; *R3*, *R6* = swamping resistances = 10,000 ohms non-inductive; *R4*, *R5* = 10,000 ohms; *R8* = 100,000 ohm non-inductive decade resistor; *C5* = 0.001 to 1.0 microfarad decade capacitance in parallel with 2,000 $\mu\mu$F air condenser. *X* = unknown; *CR* = 5 in. R. C. A. cathode ray oscillograph. *I amp.* and *E amp.* = matched capacitance coupled amplifiers with flat response from 20 to 15,000 cycles. Resistors and decades are all G. R. Co. non-inductive wound. Bridge no. 2 the same as bridge no. 1.

Figure 1 shows one of the two bridges which is an improvement of one previously described and for which operation is identical (Forbes and Landis, 1935).

Under ordinary conditions of resistance and capacitance balance is shown by a straight horizontal line, a resistance variation alone causes a tilting of the figure and a capacitance variation causes it to widen into an ellipse.

[1] I am indebted to Dr. K. S. Cole of the Department of Physiology, College of Physicians and Surgeons, Columbia University, for originally suggesting the use of the cathode ray phase angle measurement.

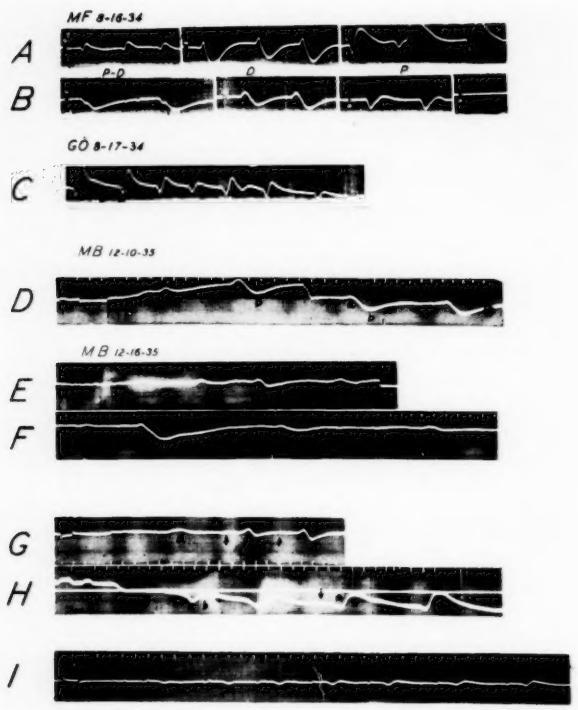

Fig. 2. A, B and C = galvanometer records from single and combined skin areas. Electrodes = zinc plates (6 cm. diam.) with gauze pads and 1/10th saturated zinc sulphate solution. *P* = palm, *D* = dorsum of hand, indifferent electrode on inner forearm, skin pierced. *P-D* = one electrode on palm and one on dorsum. Negative deflection = down for *P*, up for *D*. Original sensitivity = 1 mv. per cm. (standard signal shown). One second time lines retouched. *A* = initial response, *B* = response after 10 or 12 consecutive stimulations. Stimulus artifact can be seen in original records preceding the *a* deflection; *C* = effect of consecutive stimulation.

D, E and F = comparison of string galvanometer and potential record. Active electrode = 1 × 1½ inches zinc, zinc sulphate on palm. Inactive electrode = gauze wick in mouth, 1 mv. signal shown. E = potential amplifier and string, D and F = string galvanometer, skin potential balanced, D = initial, F = later stimulation, arrow = strong shock. Note effect of residual resistance change in D.

G, H and I = wave form with needle electrodes. Active electrode = insulated 25 gauge steel needle above elbow, 1 mv. signal shown. Arrows indicate strong shock stimulus. G and I = string record, H = potential amplifier, H and I = fall = negative. Note gradual entrance of *b* deflection in I.

Simultaneous measurements from two different skin areas were made possible by the use of two bridges, a separate cathode ray figure for each skin area being obtained by switching automatically from bridge to bridge by means of an electronic selector (Hughes, 1936). Interaction between bridges was negligible.

Simultaneous recording of endosomatic potential response with the impedance response was possible by insertion of blocking condensers C_3 and C_4, which prevented short circuiting of the slow potential response through the transformer T_2. The potential response is then seen as movement of the whole cathode ray figure up and down. The endosomatic potential variations thus recorded are subject to distortion from C_5 and the unknown C, but serve to indicate relative time relations.

Stimulation. The stimulus used was an electric shock applied through moistened pads to the finger tips of the right hand (recording electrodes on

TABLE 1

Latencies and durations

DEFLECTION	FIGURE	LATENCY	DURATION	AREA
		seconds	*seconds*	
a	2ABC	0.8–1.0	0.5 –2.0	
a	2D-I	0.6–2.0	0.8 –	
b	2ABC		2.0 –6.0	
b	2GHI		1.0 –2.0	Palm
			1.25–3.0	Dorsum
b	2DEF		1.25–7.0	Palm-dorsum or mouth-palm

left hand). It was found necessary to have the subject administer the shock himself by touching the pads in order to eliminate uncontrolled psychological stimuli (apprehension). A very light movement of the second finger sufficed to administer the shock and such movement in itself was too slight to result in an electrical skin response. The stimulus voltages used were adjusted to give a distinct shock but one felt by the subject to be bearable. The voltage was kept constant for each subject but ranged for different individuals from 90 to 180 volts from B batteries. One-half voltage ("ordinary") and full voltage ("strong") break shocks from a Harvard inductorium with 6 volts on the primary and secondary at setting *2* were also used. The finger was moistened for some two or three minutes before starting stimulation to saturate the skin and to keep the sensory shock as constant as possible (Forbes and Bernstein, 1935). Between stimulus series the fingers were remoistened.

RESULTS. A. *Endosomatic response with plate electrodes.* From 4 adult

human subjects, 2 male and 2 female, a diphasic response was obtainable from palm and from dorsum alone with great regularity. The response from palm and dorsum in series showed a variation in waveform explainable as a summation of the palmar and dorsal responses (c.f. figs. 2A and B). The diphasic waveform has been divided for convenience into an *a* and *b* deflection, as shown in the figure. The latencies and durations were variable (see table 1). The duration of the *b* wave from plate electrodes apparently varied with amplitude.

As a result of repeated stimulation, the *b* deflection gradually diminished until an apparently monophasic form resulted (fig. 2C). The monophasic form is apparently the *a* deflection remaining after the elimination of the *b* deflection. The *b* deflection may start before the *a* deflection is completed. (See apparent shortening of *a*, fig. 2A, P, compared with fig. 2B, P.)

The resultant waveform from two areas in series (P-D records) may be either diphasic, double monophasic, or apparently monophasic (figs. 2A and B, P-D records). Two plate electrodes of the inactive type about one inch apart (skin pierced) showed no response.

B. *Endosomatic response with needle electrodes.* The different waveforms with plate electrodes might still be summation effects if the indifferent area were not effectively eliminated by piercing. This, however, was not the case since similar results were obtained from three individuals using as an indifferent electrode a steel hypodermic needle thrust through the skin of the arm just above the elbow and also by using a wick electrode in the mouth where the absence of sweat glands has been held to eliminate the Tarchanoff effect. Similar records were obtained using in place of the simple string galvanometer, the balanced, high resistance input amplifier with meter or string galvanometer.

No difference in form or latency was noticeable using an indifferent needle electrode through the dorsum as compared to one above the elbow, and interchanging the placement of needle and plate along the axis of the arm did not reverse waveform. The latencies of the *a* and duration of the *b* responses with the needle electrode were again variable. (See table 1, figs. 2G, H and I.)

The use of two plate electrodes allowed distortion of the potential response by the resistance change occurring with it (fig. 2D). The needle electrode by its series capacitance effect selected out the more rapid potential phase (fig. 2G), especially of the *b* deflection. Pure potential records from mouth to palm show the same shortened period (fig. 2E) as compared to string galvanometer records (fig. 2F). It will be noted that this difference cannot be explained on a basis of greater amplitude since the small amplitude responses of figure 3F also show a longer duration. Both string and pure potential records showed a return of the *b* deflection with more intense shock stimulus (arrows in figs. 2G and H).

An impedance change occurred with both *a* and *b* potentials as shown by records on five individuals at 50 and 100 cycles on the audiofrequency

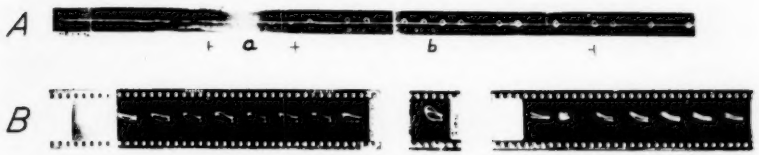

Fig. 3. A. Simultaneous impedance and potential record. Active electrode on left palm, inactive = insulated needle. A. C. Bridge with blocking condensers (C_3 and C_4, fig. 2) in circuit. Frequency 500 cycles. B = response to break shock on finger of opposite hand. Movie recording, approximately 8 frames per second.

a = negative potential, *b* = positive potential. White zero line drawn in. Unretouched frames appear lighter. Balance = C = 0.1041 μF, R = 4660 Ω. Total C variation approximately 0.003 μF.

B. Impedance response simultaneously from palm and dorsum. Stimulus = break shock to opposite hand, records with bridge of figure 2. Palm = lower figure, dorsum = upper figure; active electrode = 1 cm.^{2} zinc, zinc sulfate; inactive electrode = hypodermic needle above elbow. Simultaneous recording with electronic selector, frequency 100 cycles, continuous current stimuli. Sections are respectively 1st stimulation, residual response after 10 stimulations, and strong shock at 17th response.

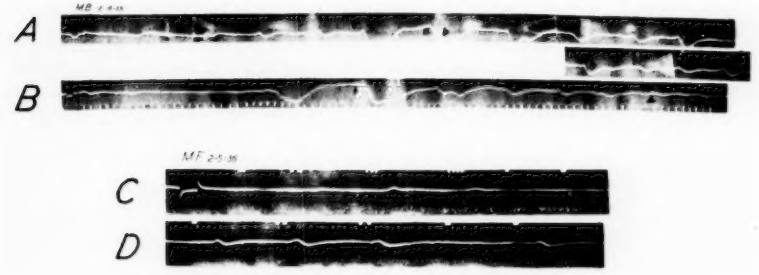

Fig. 4. A and B—effect of apprehension. Active electrode = 1 × 1½ inch zinc plate on palm; inactive = wick electrode in mouth, string galvanometer recording, successive shock stimuli. A = divided record, heavy shock occurred unintentionally at second arrow, B = similar series after accidental shock has been made impossible. Arrows = heavy shocks.

C and D—summation, palm 1 × 1½ inch zinc, to needle. Induction break shock indicated by marker, 6 volts, secondary at position 2. C = ½ strength, D = full shock (20,000 Ω potentiometer across secondary). Seconds indicated.

bridge. Figure 3A shows a widening of the cathode ray figure (impedance) beginning during the *a* and a larger change occurring during the *b* potential.

Similar records with the *a* potential only lack the marked impedance response shown in figure 3A with the *b* wave.

Palmar and dorsal skin areas show generally similar waveforms (see fig. 3B) and a similar type of impedance change. Both show impedance (widening) and resistance (tilting) changes but the palm (lower ellipse) shows the greater amplitude. A residual (not completely reversible) impedance drop occurs with repeated stimulation (fig. 3B).

C. *Anticipation and summation effects.* The *b* potential at times reentered ahead of the strong stimulation from an at first unrecognized psychological stimulus (fig. 2,I). Figure 4A shows a divided record of a continuous series with two strong shocks in the middle. The second of these was unintentional and produced some apprehension of a repetition. The *b* deflection did not diminish more than shown for a further period of several minutes. Upon rearranging the stimulus set up so as to make impossible any such accidental heavy shock, the *b* deflection diminished rapidly after a strong stimulation. (Cf. fig. 4B with 4A.) This observation has been corroborated with a similar set up intentionally produced with 3 adult subjects.

Both potentials show summation (figs. 4C and D).

Discussion. Our records show, we believe, for the first time a *predictable* potential waveform from the skin of the palm and from the dorsum separately, and indicate that the response consists fundamentally of two opposite and independent potentials from a single skin area. Previous reports of variously diphasic or polyphasic waveforms have been made (Tarchanoff, 1890; Gildemeister, 1923; Darrow, 1926; Ödegaard, 1930, and others). In these studies, however, two reacting areas were used in series and a polyphasic and variable waveform is therefore understandable. A similar technique of using a single reacting area and a non-reacting pierced area has been used without finding a predictable waveform (Darrow, 1929), but apparently a resistance variation similar to those in our figure 3D entered in some of these records to disturb the potential waveform.

It is suggested that each pure potential wave represents liberation of mediator and the slower resistance or impedance response represents the associated permeability change in membranes.

It is probable that the resistance change (permeability) is actually a second slower process and that the difference between figure 3E and F is not merely the *b* potential accentuated by a completely simultaneous resistance variation. Since $D = \frac{E}{R + 4000}$, where D is deflection, E the somatic potential, 4000 the resistance of the string, R the effective skin resistance in the range of 20,000–50,000 ohms, and $\frac{\Delta R}{R}$ is ordinarily less than 10 per cent, and since duration is roughly proportional to amplitude in

this type of record, less than a 10 per cent increase in duration can be thus accounted for.

The electrical response of the skin has been widely held to be a unitary response accompanying activity of the sudorific mechanism. (For summary, see Gildemeister, 1928, and Landis, 1932.) Our results suggest that there are two mechanisms represented and that the *b* potential is connected with a diffuse type of sympathetic activation, while the *a* potential appears non-sympathetic. Evidence for this consists of the independent variation in amplitude of the *a* and *b* potentials, their apparent separate occurrence, and the apparent dependence of the *b* deflection on an intense or an apprehension producing stimulus. The occurrence of summation of action potential has been held to indicate secretion rather than excitation in sympathetic and parasympathetic mechanisms (Rosenblueth, Forbes and Lambert, 1933), and on this basis the occurrence of summation in both *a* and *b* potentials would favor the idea of two separate reacting mechanisms.

The occurrence of a decrease of impedance with either *a* or *b* potentials is evidence that both mechanisms involve changes of tissue permeability.

There is a further suggestion of two mechanisms since both the *a* and *b* potentials show the characteristics of the "quick" potentials reported from the submaxillary gland (Rosenblueth, Forbes and Lambert, 1933). Our *a* potential is similar in latency and duration to the quick potential from single shocks to the chorda obtained by these investigators; while the *b* potential is similar in latency and form to their quick potential with tetanic stimulation of the same nerve. It differs from their slow potential in the greater steepness of the initial rise of the *b* wave as well as a tendency to a shorter duration. The magnitude of the potentials is similar in range (0.5 to 4 or 5 mv.). If both are "quick" potentials we should expect two reacting mechanisms.

The possibility that the *a* and *b* deflections are responses of the *same* mechanisms, the *a* to a single shock and the *b* to a repetitive type of stimulation from ganglia or higher centers does not seem plausible, due to the opposite polarities of the two waves.

The lag in the appearance of moisture in microphotographs (Darrow, 1932) is so great that we can draw no conclusions from the latency reported.

The *a* and *b* potentials show a superficial resemblance to smooth muscle potentials reported by Lambert and Rosenblueth (1935). However, the form is most similar to their potentials I and II, while the latencies and durations are more similar to those of potentials IIIa and IIIb even after allowance for increased conduction time. Further, our potentials *a* and *b* are on the order of millivolts, a much greater magnitude than that reported for the smooth muscle potentials. It does not seem likely, therefore, that either of the two potentials arises from smooth muscle.

The diphasic waveform apparently does not represent transmission of a

wave of activity past the two electrodes, since no reversal of waveform was obtained with reversed positions of needle and plate electrodes.

Evidence of nervous control of sweat glands other than sympathetic has been reported in monkeys (Richter, 1929) and cats (Richter and Shaw, 1930). Vasodilator effects from posterior roots are known (Kuntz, 1934), evidence for vasodilator effects from C fibers in the posterior roots has been reported by Bishop, Heinbecker and O'Leary (1933, 1936), Pinkston, Partington and Rosenblueth (1936). Schwartz (1934) found the intrinsic reflex (resistance) to be definitely sympathetic with a suggestion of a vasodilator as well as a sudorific mechanism, but did not study potentials. It is suggested that our *a* potential may be connected with one of these mechanisms.

I am indebted to Dr. C. Landis for the use of his references on the literature, to Mr. Wm. McKnight and Miss Marjorie Bolles for aid in preparing the manuscript and for technical assistance.

SUMMARY

1. Electrical response potentials were recorded from the skin of the palm and of the dorsum of the hand separately by means of a Cambridge string galvanometer with a high resistance, balanced input amplifier and an alternating current Wheatstone bridge. Simultaneous D.C. and A.C. records were also obtained.

2. The response potentials from each area were similar and consisted of a negative *a* potential followed by a positive *b* potential. The *b* potential apparently overlapped the *a*. The latencies were somewhat variable and ranged from 0.6 to 2.0 seconds for the *a* wave.

3. The *b* potential lessened in amplitude with successive stimulation. The *a* wave was obtainable alone with both weak and strong shocks while the *b* response characteristically entered with strong stimuli when they were such as to cause apprehension or excitement. Both showed summation. Individuals showed differences in the ease of reinstatement of the *b* response.

4. A predictable waveform from the skin has thus been demonstrated by recording pure potentials from a single area. The majority of "potential" waveforms previously reported are probably algebraic summations of the *a* and *b* potentials from two reacting areas, distorted by the resistance response from both areas.

5. The alternating current impedance response did not show, on strong stimulation, any change of form corresponding to the entrance of the *b* potential. An impedance variation was asociated with each potential.

6. The sympathetic sudorific mechanism is known to be the source of an electrical potential and resistance response of the skin. The *b* potential shows the diffuse sympathetic character to be expected from sudorific

activation but it is suggested that the *a* potential represents a second, somewhat independent nervous response mechanism. Independence of the two waves, similarity to "quick" glandular potentials found by other workers, and the apparent dependence of the *a* and *b* responses upon different types of stimulating conditions favor this view.

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SURVIVAL OF THE ADRENALECTOMIZED NEPHRECTOMIZED RAT

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It has been proved that the increased excretion of sodium and chloride after adrenalectomy in dogs, cats and rats is of fundamental importance. The loss of electrolyte precedes the terminal syndrome of adrenal insufficiency. Both Loeb (4) and Harrop (1) have explained their experimental results by an hypothesis which assumes that the essential action of cortin is brought about by its effect on the kidney itself. If the action of cortin were confined to its effect on the kidney, the adrenalectomized animal, during its survival after nephrectomy, should not manifest the symptoms of adrenal insufficiency, and the administration of cortin should not benefit such an animal. Three years ago one of us (Ingle) noted that the anesthetized working rat showed a longer survival after nephrectomy than it did after adrenalectomy. After both adrenalectomy and nephrectomy the survival period was shortened to a few hours. Although this result is contrary to the hypothesis in regard to the place of action of cortin, the experiment is not conclusive because of the shock from the operation and the abnormal condition which is produced by the accumulation of toxic products. In the present study the effects of cortin on the survival of the adrenalectomized, nephrectomized animal have been determined.

METHOD. Adult male rats were closely matched, on a basis of age and weight, into groups of three. One animal of each group had only the kidneys removed. A second had both the kidneys and adrenals removed and received subcutaneous injections of cortin every eight hours. A third had both the kidneys and adrenals removed but did not receive any treatment. All operations were performed in a single stage. Aseptic precautions were not taken. Ten groups of animals, which will be termed the "non-work" series, were kept in the usual housing cages with an excess of food and water until death. Ten additional groups of animals ("work" series) were anesthetized with phenobarbital sodium, and immediately following operation the gastrocnemius muscle was stimulated to lift a 100 gram weight three times per second until death. The methods employed have been described previously in detail (2, 3).

RESULTS. In the "non-work" series, the average period of survival of

the nephrectomized animals was 89 hours, with a range of 58 to 130 hours. The adrenalectomized nephrectomized animals treated with cortin showed an average period of survival of 86.7 hours, with a range of 56 to 136 hours. The periods of survivals of the untreated adrenalectomized, nephrectomized animals were much shorter with an average of 19.5 hours and a range of 5 to 45 hours. The amount of urea in the blood of some of the nephrectomized animals at time of death varied from 450 to 974 mgm. per 100 cc.

The effect of work and anesthesia was to shorten the average time of survival to less than that seen in the "non-work" series. Those animals subjected only to nephrectomy had an average period of survival of 52.6 hours with a range of 34 to 65 hours. The adrenalectomized, nephrecto-

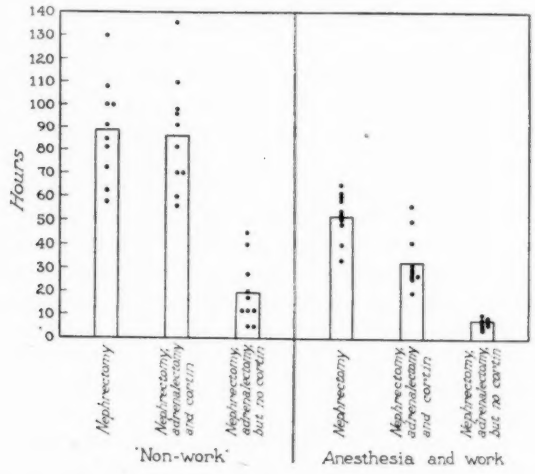

Fig. 1. Effect of cortin on the performance of the adrenalectomized, nephrectomized rat.

mized animals which were treated with cortin had an average period of survival of 33.7 hours, with a range of 20 to 56 hours. The untreated adrenalectomized, nephrectomized animals had an average survival of 7 hours, with a range of 4 to 10 hours. Examination of the total work records in this series showed that the best performance of any untreated, adrenalectomized, nephrectomized rat was still far inferior to the poorest performance among the treated group. The best average performance was shown by those nephrectomized animals whose adrenal glands were left intact. The results are summarized in figure 1.

COMMENT. Removal of the kidneys, which closes the most important avenue for loss of salt from the body, does not prevent death from adrenal

insufficiency. The administration of cortin has a striking effect in prolonging the survival of the adrenalectomized, nephrectomized animal. Adrenalectomized, nephrectomized rats may be conveniently used in the work test (3) to determine the presence of activity in extracts of the adrenal cortex. By this test the presence or absence of activity in an extract can be determined within twelve hours. None of numerous tests have been found in error by subsequent evaluation by other reliable methods. No attempt has been made to standardize this test for quantitative assay.

The periods of survival of the animals of the "non-work" series which were treated with cortin compared favorably with the periods of survival of similar animals which had not undergone adrenalectomy. In the "work" series the survival time of the animals treated with cortin remained less than the survival time of those animals whose adrenals were intact. Under conditions of great stress the inferiority of the animals treated with cortin may only reflect the inability of intermittent injection of the hormone to duplicate the full effect of the continuous secretion by the glands themselves.

It seems probable that the action of cortin is not confined to any single tissue. It will act in the absence of the whole gut, the pituitary body, the thyroparathyroid apparatus, the thymus, the gonads, and after nearly complete removal of the pancreas. Lesions in any part of the hypothalamus do not prevent its beneficial effect in the adrenalectomized animal.

SUMMARY

The periods of survival of nephrectomized rats were compared to periods of survival of adrenalectomized, nephrectomized rats with and without cortin treatment. Ten groups of animals were anesthetized and the gastrocnemius muscle stimulated to contract three times each second until death. A second series of animals was studied under "non-work" conditions. It has been demonstrated that the administration of cortin markedly prolongs the time of survival of adrenalectomized, nephrectomized rats under ordinary living conditions and under conditions which include work and anesthesia.

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EFFECT OF EPINEPHRINE ON GLUCOSE EXCRETION IN FASTED DEPANCREATIZED DOGS

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Probably the strongest evidence in support of gluconeogenesis from fatty acids was that presented by Chaikoff and Weber (1) showing that epinephrine injected into starving depancreatized dogs resulted in the excretion of more glucose than could be accounted for from all sources other than fatty acids. Bollman, Mann and Wilhelmj (2) repeated this work and found that the extra glucose excreted was well within the limit of carbohydrate stores. In fact, by determining muscle glycogen before and after epinephrine administration they found that the extra glucose was equal to the amount of glycogen removed from the muscles during this period. Chambers, Himwich and Kennard (3) also consider muscle glycogen as the source of the extra glucose excreted upon epinephrine administration. Because of the discrepancies in the results reported, the experiment was repeated as originally described by Chaikoff and Weber.

METHOD. Fourteen female dogs were completely depancreatized and placed on a stock diet to which fresh ground pancreas (200 gm. per day), sucrose and milk was added. Insulin was given subcutaneously with each meal in sufficient amounts to keep the blood sugar below the renal threshold level. When the animals were in good nutritional condition, food and insulin were withdrawn. The fasting period was considered as starting at the time of the first omitted meal. At the end of the second day of fasting, the bladders were emptied by catheter and urine was collected under toluene for three consecutive twelve-hour periods. Catheterization was performed at the end of each period. One cubic centimeter of 1:1000 epinephrine was injected subcutaneously every three hours of the last period.

Urinary glucose was determined by the Shafter-Hartman method, nitrogen by the macro-Kjeldahl. Calculations were made as follows using values determined by Chaikoff and Weber. Liver glycogen: The liver was taken as 5 per cent of the body weight and was considered as containing 0.35 per cent glycogen (the maximum amount of glycogen found by Chaikoff and Weber in the liver of a fasted depancreatized dog after three

days of fasting). Protein: Each gram of nitrogen excreted during the epinephrine period in excess of that excreted during the previous twelve hours was considered a possible source of 6.4 grams of the "extra" glucose. Glycerol from fat: Chaikoff and Weber found that the caloric expenditure of the dogs during the twelve hour epinephrine period was 50 calories/kilo. If the dog was utilizing only fat for energy, the glycerol from this fat would

TABLE 1

DOG NUMBER	WEIGHT	LAST 12 HOUR PERIOD BEFORE EPINEPHRINE			1 CC. 1:1000 EPINEPHRINE SUBCUTANEOUSLY AT 3 HOUR INTERVALS				MUSCLE GLYCOGEN	TOTAL AVAILABLE SOURCE OF EXTRA GLUCOSE AS GLUCOSE
		Glucose	Nitrogen	D:N	Glucose	Nitrogen	D:N	Extra glucose		
	kilo	*grams*	*grams*		*grams*	*grams*		*grams*	*grams*	
1	8.8	6.8	2.62	2.60	25.9	4.03	6.42	19.1	16.5	31.7
4	8.3	1.22	1.07	1.14	10.20	1.38	7.38	8.98	15.6	23.43
5	3	0.07	0.48	0.15	5.19	2.66	1.95	5.12	5.6	21.6
6	5.7	4.06	2.27	1.79	14.76	3.61	4.09	10.7	10.7	20.7
7	5	3.80	1.56	2.4	9.9	1.28	7.7	6.1	10.2	13.9
8	9.1	3.25	1.02	3.08	28.08	3.39	8.28	24.83	17.1	38.8
9	7	0.98	1.09	0.9	26.22	3.80	7.0	25.24	13.7	36.2
10	7	10.88	2.76	3.93	29.66	3.25	9.13	18.78	18.8	28.9
11	10	5.66	1.91	2.96	16.17	3.26	4.96	10.51	13.2	26.7
12	8	4.88	1.61	3.04	11.87	2.15	5.53	6.99	15.0	24.2
13	Samples lost				12.01	2.12	5.65		17.1	23.52
14	9.1	9.33	2.99	3.55	31.92	4.18	7.64	22.59	17.1	38.8
15	10	6.12	1.72	3.55	20.30	2.45	8.28	14.18	18.8	28.9
16	6.8	3.72	1.39	2.66	17.43	1.88	9.29	13.71	12.8	20.6
Average.....		4.80	1.71	2.44	18.63	2.84	6.63	13.16	13.9	25.8

TABLE 2

OBSERVERS	NUMBER OF DOGS	PIRE-EPINEPHRINE D:N	NITROGEN EXCRETED DURING EPINEPHRINE PERIOD	GLUCOSE EXCRETED, EPINEPHRINE PERIOD	"EXTRA GLUCOSE", EPINEPHRINE PERIOD
			grams/kgm.	*grams/kgm.*	*grams/kgm.*
Chaikoff and Weber.....	7	6.5	0.41	4.9	3.1
Bollmann, Mann and Wilhelmj.....	4	3.2	0.39	2.2	1.2
This report.....	14	2.4	0.41	2.4	1.8

be a possible source of 0.53 gram per kilo of body weight. Muscle glycogen: If the muscles constitute 40 per cent of the body weight and contain at this time 0.47 per cent glycogen (determined by Chaikoff and Weber), muscle glycogen is capable of contributing 1.88 grams glucose/kilo body weight. "Extra" glucose was determined by subtracting the glucose excreted during the period just prior to the epinephrine period from the

glucose excreted during the epinephrine period. For example, dog 6 weighed 5.7 kilo, excreted 14.76 grams glucose with epinephrine stimulation and 4.06 grams in the period just previous or extra glucose amounting to 10.7 grams. Calculating the possible glucose from the aforementioned sources we find that liver glycogen may contribute 0.05 × .0035 × 5,700 or 0.99 gram, glycerol may contribute 5.7 × 0.53 or 3 grams, protein (3.61 − 2.27) 6.4 or 1.34 × 6.4 or 8.58 grams, and muscle glycogen 1.88 × 5.7 or 10.7 grams.

Results. As can be seen from table 1, in no case did the amount of "extra" glucose exceed the total possible source of glucose from sources other than fatty acids. In most cases the extra glucose could be accounted for either by glycogen alone, or by protein, glycerol and liver glycogen combined.

Discussion. These results do not check with those obtained by Chaikoff and Weber. One point that may contribute to this discrepancy is the great difference in D:N ratio for the period immediately preceding the epinephrine stimulation (table 2). The average D:N ratio for this period as reported by Chaikoff and Weber was 6.5 while our average D:N ratio for the same time was 2.4. The amount of nitrogen in both cases is similar and the difference all lies in the glucose excretion which would indicate that Chaikoff and Weber's animals had not reached a fasting level and that glycogen stores were contributing a good share of the "extra" glucose.

Our "extra" glucose is almost equal to the average possible muscle glycogen, confirming Bollman, Mann and Wilhelmj whose results showed that muscle glycogen may possibly be the source of this "extra" glucose. It is interesting to note how closely the nitrogen values check in the three studies.

CONCLUSION

These experiments show that it is not necessary to postulate gluconeogenesis from fatty acids to account for the extra glucose excreted under the influence of epinephrine, and second, the average amount of extra glucose excreted was not more than could be derived from muscle glycogen as computed.

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GLOMERULAR FILTRATION AND UREA EXCRETION IN RELATION TO URINE FLOW IN THE DOG

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Of chief historical interest in the study of the excretion of urea by mammals are the investigations of Austin, Stillman and Van Slyke (1921) and of Moeller, McIntosh and Van Slyke (1928) on man, which introduced the empirical concept of standard and maximum urea clearances. This concept was accepted for dogs by Jolliffe and Smith (1932a, b) and by Summerville, Hanzel and Goldblatt (1932), and it has been assumed in the recent studies of Van Slyke, Rhoades, Hiller and Alving (1934). Dominguez (1935) collected from the literature data on urea clearances in the dog and, adding some further observations furnished by Goldblatt, formulated an equation to describe changes in the urea clearance relative to the rate of urine formation. This equation was applicable at all urine flows, as opposed to the discontinuity of function implicit in the concept of standard and maximum clearances. Dominguez' treatment was also empirical, the fusion of standard and maximal clearances effected by him being accomplished by the arbitrary assumption that the maximum clearance of Van Slyke could be taken as the asymptote towards which the urea clearance approached as the urine flow increased, and which was approximated at the augmentation limit. This type of analysis is of uncertain value, since the indiscriminate admixture of data without regard for the conditions under which they have been obtained is apt to obscure important physiological variables. In 1925 Bourquin and Laughton presented data on dogs that could not be explained in terms of standard and maximum clearances. They studied cycles of diuresis and showed that, at any urine flow, when the urine flow was rising rapidly the urea clearance was usually higher than when the urine flow was falling. Their experiments were complicated, however, by the unacknowledged error of dead space, and also by a possibly inadequate method of emptying the bladder.

The present observations on the excretion of urea in the dog were undertaken with the hope that it might be possible to define more exactly the several factors operating in this important process. The central feature in this problem is the measurement of the rate of glomerular filtration.

Recent investigations in this laboratory, starting with the work of Jolliffe, Shannon and Smith (1932), have furnished the experimental basis for the use of the non-metabolized carbohydrate, inulin, for this purpose in the dog-fish (Shannon, 1934), the dog (Shannon, 1935) and man (Shannon and Smith, 1935). Supplementary evidence supporting this procedure has been furnished by Professor Richards and his co-workers, who were led independently by their experiments on the frog kidney to the study of the excretion of inulin in the dog (Richards, Westfall and Bott, 1934, 1936, Westfall and Hendrix, 1936).

The parenteral administration of inulin involves, however, the injection of relatively large quantities of saline, and its use is therefore sometimes disadvantageous in experiments in which one wishes to avoid any derangement of salt and water balance in the body. For this reason the creatinine clearance, which we have shown to be identical with the inulin clearance in the normal dog (Shannon, 1935, 1936) has been used as a measure of the filtration rate. The identity of the simultaneous inulin and creatinine clearances at low urine flows, where the U/P ratio is as high as 570, and data presented in this communication (fig. 5) lead us to believe that no error due to back diffusion of creatinine is introduced in the present experiments. (The observations of Van Slyke, Hiller and Miller (1935) on the excretion of ferrocyanide in the dog have been interpreted as supporting the belief that the creatinine clearance is at the level of glomerular filtration, but the great variability of the published results makes them of uncertain value. The standard deviation of the 27 creatinine-ferrocyanide comparisons was 18 per cent of the mean value of 0.96, a variability too large to indicate with certainty that there is no significant difference between these clearances.)

Experimental. We have proceeded on the assumption that different dogs might behave in a different manner with respect to the excretion of urea, and have therefore examined every dog completely, i.e., in respect to all variables. And on the further assumption that the same urine flow on different occasions might not be physiologically equivalent, even in the same animal, our experiments, unless otherwise indicated, have been so designed that the urine flow has varied throughout the entire physiological range in each series of observations. Each experiment has been so conducted that the rate of urine flow should return at the conclusion of the experiment, or at least once during the experiment, to the initial rate, or as near to the initial rate, as possible. (See table 1.) We believe that failure to observe these conditions is in part responsible for a misrepresentation of the true relation between the rate of water excretion and the urea clearance.

The urea and creatinine clearances have been examined in over 800

periods in 6 dogs. These animals were well trained females weighing from 17 to 28 kgm. Three of the dogs were observed upon a cracker meal, sucrose and lard maintenance diet, two upon a mixed diet, and one dog on both of these diets at different times. Adequate vitamins were given to all animals, and a salt mixture was used to supplement the low protein diet.

The general experimental procedures and chemical methods were the same as those that have been reported previously from this laboratory,

TABLE 1

A typical experiment (no. 37B, dog E, low protein diet) to illustrate the routine of the experiments performed on stationary and falling urine flows

Water was withheld from the animal for the 48 hours preceding the experiment. One hundred, 50, 50 and 50 mgm. per kgm. of creatinine were injected subcutaneously at the zero hour, 2:55, 5:14 and 7:44. Fifty and 15 cc. of water per kgm. were administered by stomach tube at 2:05 and 7:14.

PERIOD	TIME FROM ZERO HOUR	DURATION OF PERIOD	URINE FLOW	PLASMA CONCENTRATION		*U/P* RATIO		CLEARANCE		UREA / CREATININE CLEARANCE RATIO
				Urea	Creatinine	Urea	Creatinine	Urea	Creatinine	
		minutes	*cc./minute*	*mgm. per cent*	*mgm. per cent*			*cc./minute*	*cc./minute*	
1	1:00–1:30	30	0.136	25.6	9.62	171.0	426.0	23.2	58.0	0.400
2	——2:05	35	0.143	25.6	9.84	164.0	416.0	23.4	59.5	0.393
3	3:10–3:40	30	4.10	23.7	11.4	8.64	15.4	35.5	63.1	0.563
4	——4:11	31	4.30	22.5	11.8	8.28	14.5	35.6	62.4	0.570
5	——4:40	29	4.10	21.3	11.9	8.44	15.1	34.6	61.9	0.559
6	——5:11	31	3.80	20.1	10.9	8.63	15.8	32.8	60.0	0.547
7	——5:43	32	3.03	19.0	9.4	10.6	19.6	32.1	59.3	0.541
8	——6:14	31	2.87	18.1		11.3		32.4		
9	——6:44	30	1.40	17.6	12.1	21.2	41.9	29.7	58.7	0.506
10	——7:17	33	0.88	17.2	12.1	31.6	66.1	27.8	58.2	0.478
11	——7:44	27	0.48	16.9	11.9	54.4	122.0	26.1	58.4	0.446
12	——8:14	30	0.250	16.8	10.3	98.8	237.0	24.7	59.2	0.417
13	9:20–9:50	30	1.63	15.7	11.4	19.6	37.9	32.0	61.8	0.518
14	——10:20	30	1.03	15.4	11.3	29.3	58.5	30.2	60.3	0.501

except in the following respects. In all instances where the rate of urine flow was below 2 cc. per minute, the bladder was washed out, as described by Shannon (1936). In some cases the creatinine determinations were done on iron filtrates (Steiner, Urban and West, 1932) of plasma and urine, and in others on diluted urines and tungstic acid filtrates of plasma as detailed by Shannon, Jolliffe and Smith (1932). In order to maintain the concentration in the plasma as constant as possible creatinine was administered in 8 to 10 per cent solution injected subcutaneously in circum-

scribed areas in doses of 50 to 100 mgm. per kgm. every 2.5 to 3.5 hours.[1] In the calculation of creatinine clearances no correction has been applied for endogenous creatinine; this correction would in any case be slight with plasma levels of 10 mgm. per cent, and constant for all periods.

EXPERIMENTAL RESULTS. For convenience of description the experimental results will be discussed as of two types. In the first type, observations were made while the urine flow was either constant or falling, the observation periods being removed at least one hour from the administration of water. The second type of experiment consists of observations in which the effect of rising urine flow was examined against a background afforded by the first type of experiment.

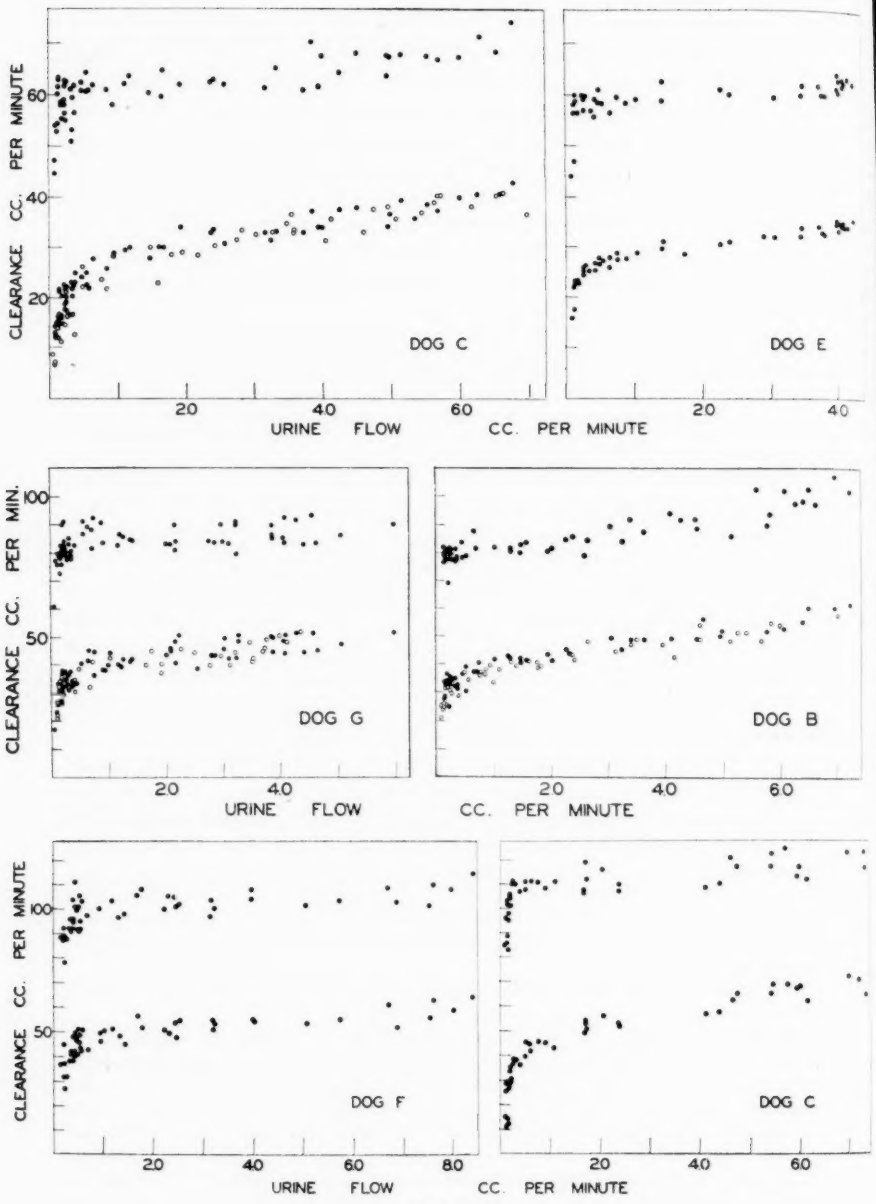
Observations on falling urine flows: Creatinine clearance. A typical experiment belonging to the first type is reported in full in table 1. Graphical summaries of all experiments of this first type (except those on dog A[2]) are given in figures 1 to 3, where the urea and creatinine clearances are plotted against the rate of urine formation.

The first significant fact demonstrated by these experiments is that the rate of glomerular filtration is essentially unrelated to the rate of water excretion within the range of ordinary experimental urine flows. This fact is revealed to better advantage by the summary of all the creatinine data given in figure 4. Each datum in the figure represents the mean of five clearance periods, selected by urine flow. For ease of comparison, the data on each dog have been adjusted to a percentage basis, taking the mean creatinine clearance at 0.5 to 1.0 cc. per minute as 100 per cent. It will be observed in figure 4 that between 0.5 and 4.0 cc. per minute the average creatinine clearance is essentially constant.[3] At rates of urine formation above 4.0 cc. per minute there is a variable tendency for the filtration rate to increase. The maximal increase (relative to the rate at 0.5 cc. per minute) in the various dogs shown in this figure is 25 per cent for dog B, 13 per cent for dog C on low protein diet (dots) and 6 per cent on high protein diet (circles), 6 per cent for dog E, 8 per cent for dog F, 3 per cent for dog

[1] Any period following a large subcutaneous injection may show an aberrant urea clearance. For this reason we have administered creatinine dissolved in minimal quantities of water.

[2] No creatinine clearances were done on dog A, but the urea clearances would fall within the scatter of those of dog C on a low protein diet.

[3] There is one significant fact that is lost when the creatinine clearances are averaged in the manner shown in figure 4. The first one or two periods at the peak of diuresis may be slightly higher than the mean value of the clearance when the urine flow is between 0.5 to 4.0; cc. per minute. This phenomenon is inconstant, and may appear regardless of where the peak of diuresis may be (i.e., 1 cc. or 8 cc. per minute). This may be similar to the phenomenon described by Jolliffe, Shannon and Smith (1932), which was attributed in part to the act of administration of water, as well as to excessive hydration when large volumes of water are given to obtain high urine flows.


CLEARANCE CC. PER MINUTE
60
40
20
DOG C
DOG E
20
40
60
URINE FLOW CC. PER MINUTE
CLEARANCE CC. PER MIN.
100
50
DOG G
DOG B
20
40
60
URINE FLOW CC. PER MINUTE
CLEARANCE CC. PER MINUTE
100
50
DOG F
DOG C
20
40
60
80
URINE FLOW CC. PER MINUTE

Figs. 1–3

G. These changes in filtration rate are relatively small in contrast to an 800–1600 per cent increase in the rate of water excretion. Conversely, when the urine is below 0.5 cc. per minute the filtration rate tends to fall.[4] These changes in glomerular filtration, because of their variability and the circumstances required to evoke them, may best be interpreted as due to incidental changes in renal circulation, or to excessive hydration or dehydration of the blood, rather than to a specific reaction of the glomerular apparatus. A possible exception to the above conclusion is evident in dog B, as shown in figure 2, which consistently showed a significant increase in the creatinine clearance with increasing urine flow on every occasion that it was examined. But even here the change in creatinine clearance is of a much smaller order of magnitude than the change in the rate of water excretion, and cannot be held to bear a causal relation to the latter.

Concerning the conclusion that the rate of water excretion is essentially independent of the rate of glomerular filtration, it may be noted that Peters (1935) has criticized this view and asserted that it was not warranted by any available evidence. We believe, however, that our present data are adequate to justify the assertion that in water diuresis in the dog there is no functional relationship between the rate of glomerular filtration and the rate of urine flow. This conclusion is based chiefly upon two facts. The first is the point made above, that in some animals wide variations in urine flow are not accompanied by significant changes in glomerular filtration. Second, in spite of the fact that under certain conditions changes in glomerular filtration may be accompanied by proportional changes in urine flow (thus keeping the U/P ratio of creatinine constant with small

[4] We believe that the fall in the creatinine clearance at low urine flows is not due to back diffusion of creatinine, since we have demonstrated that the creatinine and inulin clearances remain identical at these same U/P ratios (Shannon, 1936). Moreover, when the creatinine clearance is depressed, because of excessive dehydration, and this dehydration is relieved by the administration of moderate amounts of water, the creatinine clearance returns to the general level with no essential change in the creatinine U/P ratio (see fig. 4).

Figs. 1–3. Creatinine and urea clearances in relation to urine flow, as observed when the latter is stationary or falling.

The data shown in these figures are the experimental observations that are further analyzed in figures 4 and 6. Each point is a single clearance period uncorrected for surface area. In each figure the upper data represent creatinine clearances, and the lower data represent urea clearances; the circles represent urea clearances in the absence of administered creatinine, the dots, urea clearances in the presence of administered creatinine. The data on dog C (fig. 1) and dogs E and B were obtained on a low protein diet, while those on dog C (fig. 3) and dogs F and G were obtained on a mixed diet.

The presence of exogenous creatinine in no way disturbs the relation of the urea clearance to urine flow, as observed without exogenous creatinine. (See dog C, fig. 1, dogs G and B, fig. 2.)

absolute changes in urine flow resulting), it is also true that wide changes in the former are sometimes not accompanied by any change in urine flow whatever (see fig. 5).

Urea clearance. In contrast to the constancy of the creatinine clearance, we find that the urea clearance increases systematically with the rate of urine formation throughout the entire range of urine flow, and no point may properly be designated as an augmentation limit, either in the sense of

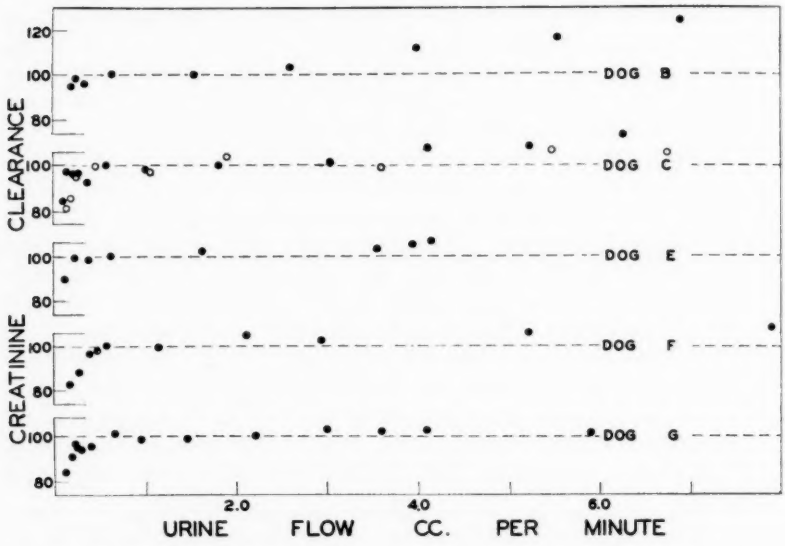


Fig. 4. Creatinine clearances (glomerular filtration) in relation to rate of water excretion.

The creatinine clearances of each dog, shown in figures 1 to 3, are averaged in groups of 5 according to urine flow and expressed on a per cent basis, taking the average values observed in each dog at urine flows of 0.5 to 1.0 cc. per minute as 100.

The creatinine clearance, which in the dog may be taken as equal to the rate of glomerular filtration, is essentially unrelated to urine flow between 0.5 and 4.0 cc. per minute. The decrease observed at urine flows below 0.5 cc. and the increase observed at urine flows above 4.0 cc. are probably referable to changes in circulation, blood hydration, etc., rather than to a specific reaction of the glomerular apparatus.

Austin, Stillman and Van Slyke (1921) or of Dominguez (1935). Examination of different dogs reveals that the rate at which the urea clearance changes with changing urine flow is quantitatively not the same in all animals. The rate of change is greatest in dog C (on both a low and high protein diet) and least in dog F (high protein diet).

The administration of creatinine does not alter the relationship between urea clearance and urine flow, as is demonstrated by the data on three

dogs, C, B, and G in figures 1 and 2. In view of this fact, we believe that observations on the urea and creatinine clearances made in experiments

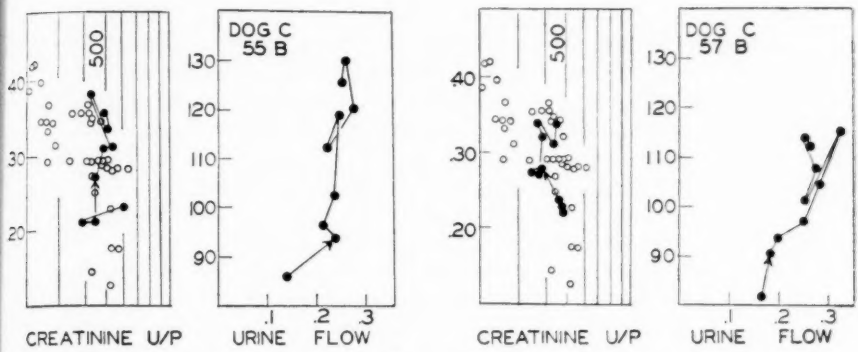


Fig. 5. Experiments showing the effect of slow hydration of a dehydrated dog on the creatinine and urea clearances, and on the urea/creatinine clearance ratios.

The relation between urea/creatinine clearance ratio and the log of the U/P ratio of creatinine is shown at the left in each instance. The open circles are the data shown for dog C in figure 4, as observed on a stationary or falling urine flow. The solid dots represent observations on a rising urine flow. The figures to the right relate creatinine clearance to urine flow in the same manner as in figures 1 to 3. The urine collection periods in these experiments were approximately 40 minutes in duration.

Experiment 55 followed 24 hours' abstinence from water. After each of the first 5 periods 50 cc. of water were injected subcutaneously.

Experiment 57 followed 48 hours' abstinence from water. After two control periods, 100 cc. of water were injected subcutaneously, and after each of the next 3 periods, 50 cc. of water were injected in the same manner.

During dehydration, the urea/creatinine clearance ratio appears to be depressed, relative to the "normal" values observed on a falling urine flow. Not only is the urea/creatinine clearance ratio depressed during dehydration, but the creatinine clearance itself is depressed below the "normal" values, as observed on a falling urine flow (by hemoconcentration, etc.). When hydration is effected slowly by the subcutaneous injection of water, both the creatinine clearance and the urea/creatinine clearance ratio rise; there is no great change in the U/P ratio of creatinine, and consequently little increase in urine flow. The fact that the creatinine clearance can increase over 30 per cent without a significant change in urine flow strengthens the belief that the rate of water excretion is within wide limits independent of the rate of glomerular filtration.

The rise in the urea/creatinine clearance ratio shows that the urea clearance increases to a much greater extent during slow hydration than does the creatinine clearance. This phenomenon is not the same as the exaltation of the urea clearance shown in figures 7 and 8.

where creatinine has been administered are applicable to the normal animal uncomplicated by creatinine injection.

Assuming that urea is freely filterable at the glomerulus (an assumption

for which there is available a large amount of evidence which need not be reviewed here) then the urea clearance should be equal to the rate of filtration unless some of the filtered urea is destroyed or reabsorbed by the renal tubules. It is well known that the urea clearance is lower than the glomerular clearance, presumably for one or both of these reasons; since there is no evidence to the contrary we will assume in the following discussion that the deficit in the urea clearance is due only to tubular reabsorption. And since the creatinine clearance in the dog may be taken as equal to the rate of glomerular filtration, the degree to which urea has been reabsorbed is indicated by the fraction 1.0—urea/creatinine clearance ratio, while the U/P ratio of creatinine indicates the degree to which the glomerular filtrate has been concentrated by the reabsorption of water. The relationship between urea reabsorption and water excretion is therefore best portrayed, for our present purpose, by plotting the urea/creatinine clearance ratio against the U/P ratio of creatinine, using the logarithm of the latter for convenience, since this term varies from 10 to nearly 800. The data presented in figure 6 are analyses made in this manner of the data shown in figures 1 to 3. (Attention is again called to the fact that these observations were all made under conditions where the urine flow was constant or falling.)

Three points can be established from these data. First, the urea clearance varies with urine flow in a systematic manner throughout the entire range of the latter. Although the relationship between these two variables differs considerably from one dog to another, the general trend between urea clearance and urine flow is the same in all dogs.[5] Second, the degree of urea reabsorption at a given urine flow in a given dog is quantitatively the same when the rate of glomerular filtration is low (on a low protein diet) and when the rate of glomerular filtration is high (on a high protein diet). This fact was demonstrated with particular care in dog C. Third, at the highest urine flow obtainable (about 8 cc. per min.) a large fraction of the filtered urea is still reabsorbed.

So far as the first of the above points is concerned, we suppose that the essential factors in urine flow are the duration of time during which the concentrated urine is in contact with the tubules distal to the point of water reabsorption, and the tendency for the contained urea to diffuse outward.

[5] The above description does not deny the general parallelism that has previously been noted in this and other laboratories between the urea and creatinine clearance under ordinary experimental conditions. In most reported experiments the urine flow has been relatively large, under which conditions there would be little variation in the urea/creatinine clearance ratio. The progressive increase in urea reabsorption noted here would not in any case be demonstrable except in experiments in which the changing urine flow was followed in a systematic manner. But where the urine flow is varied over a wide range, the urea/creatinine clearance ratio, or any measurement implied by this ratio (such as urea extraction ratio or urea clearance) must undergo wide variation in relation to urine flow.

Both these factors can be related to the U/P ratio of creatinine, and we note that the extent of urea reabsorption is related to the logarithm of this term. This relationship, with the exceptions noted below, appears to obtain in all dogs whether the urine is constant or falling, and regardless of diet, etc. For convenience we will refer to it as the logarithmic relationship, without implying any rigid mathematical description.[6]

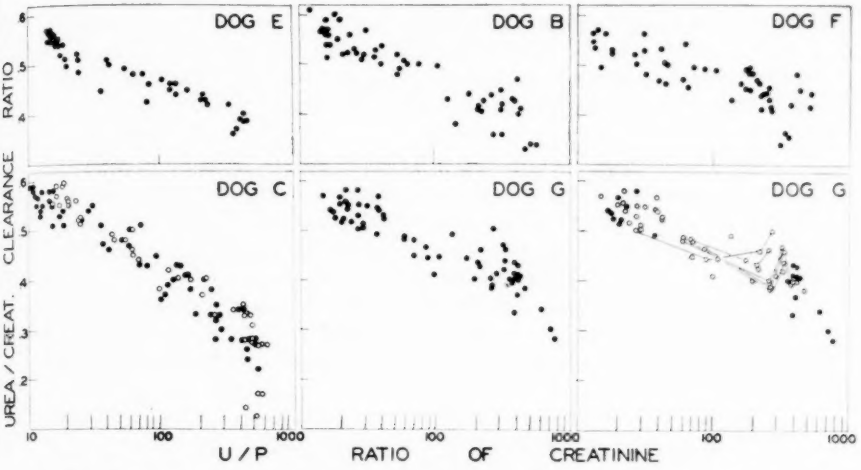


Fig. 6. The urea/creatinine clearance ratio in relation to the reabsorption of water, as indicated by the U/P ratio of creatinine.

Data as in figures 1 to 3.

The fraction of filtered urea that is reabsorbed is equal to 1.0—the urea/creatinine clearance ratio. The relationship shown here is apparently linear and indicates increasing urea reabsorption with increase in water reabsorption throughout the entire range studied. Diet in no way influences this relationship, as demonstrated specifically in the case of dog C.

At high urine flows the urea/creatinine clearance ratio is less than 0.6; if extrapolation is made to a U/P ratio of 1.0, this ratio would have a value no higher than 0.7. In the data of dog G, observations made at stationary urine flows are indicated by dots, and observations on a falling urine flow are indicated by circles. In 5 experiments, consecutive periods have been connected by lines.

Further inspection of figure 6 reveals that the scatter of the data is greater than is to be expected from experimental error, indicating that urea

[6] This relationship is usually as evident in single experiments as in the mass plots shown in figure 6. An exception to this statement was observed in dog G. Here in some experiments the depression of the urea clearance on falling urine flows appeared to be related to the rate of deceleration of the urine flow, rather than the existing creatinine U/P ratio. But examination of the remainder of the data on this dog and of data obtained on the others did not support the suggestion that the rate of deceleration of the urine flow is itself a factor influencing urea reabsorption.

reabsorption may be affected by one or more factors other than those involved in urine flow. One such factor is dehydration. In dogs that have been deprived of water for 24 hours or more the urea clearance may be excessively depressed (i.e., below the "normal" value for a given urine flow). An instance of this nature is given in figure 5. This excessive depression can be corrected by administering water in such a manner (preferably subcutaneously) that the rate of urine formation is not increased (at least to no greater extent that can be accounted for by the accompanying increase in glomerular filtration, as shown in fig. 5). We call attention to this phenomenon at this time, without wishing to discuss it further, because it accounts in part for the broad scatter of the data at low urine flows.

In interpreting the logarithmic relationship shown in figure 6, it should be recognized that the U/P ratio of creatinine might be physiologically related to *1*, the degree of hydration of the body cells, or of the renal tubules, as well as to *2*, the concentration of hormones, proteins, etc., in the plasma. And this ratio is at least roughly indicative of *3*, the extent of water reabsorption by the tubules; *4*, the rate of fluid movement through the tubules, following water reabsorption and *5*, the potential extent to which urea would have been concentrated in the tubules had there been no urea reabsorption.

It seemed to us that it should be possible to rule out of consideration some of these factors. For example, to induce diuresis (*3*, *4* and *5*) independently of hydration and blood composition (*1* and *2*), we have used the intravenous administration of 50 per cent sucrose solution, with and without the simultaneous administration of pitressin. An experiment of this type is shown in figure 8, experiment 84B.[7] In this and other such experiments the logarithmic relationship has been maintained, from which we conclude that it depends upon *3*, *4*, and *5*, or upon some other local renal condition, rather than upon the systemic factors.

Observations on rising urine flows. It was early observed that the urea clearance tends to rise to abnormally high values, relative to the creatinine clearance, when the urine flow is increasing rapidly. A series of experiments examining this phenomenon in relation to water diuresis is given in figure 7. This figure includes experiments illustrating the extent to which the rate of acceleration of the urine flow, the original rate of urine flow, and the amount of water given, are related to this exaltation of the urea clearance.

[7] This does not imply that systemic factors have no influence upon urea reabsorption, as has been indicated by the observations of others (see Poulsson, 1930 and the experiments reported here, particularly experiment 85B, fig. 8). The point under discussion concerns only whether or not these factors (in contradistinction to local renal factors) are responsible for the progressive increase in urea reabsorption that is related to decreasing urine flow.

If the urine flow is increased slowly, the logarithmic relationship previously described may be exactly retraced. This is demonstrated in experiment 26B, where the rate of urine formation was caused to increase from 0.14 cc. to 3.74 cc. per minute by the slow administration of water over a period of seven hours. But in 18 attempts this was the only instance in which so slow an acceleration of the urine flow could be obtained.

With more rapid acceleration of urine flow experiment 28B was obtained. Although, in this instance, the logarithmic relationship was approximately retraced, there is an evident tendency for the urea clearance to rise, relative to the creatinine clearance, during the early part of the acceleration of urine flow.[8]

The most frequent result obtained on an increasing urine flow is of the nature of that shown by experiment 70B and table 2; the urea clearance rises markedly, relative to the creatinine clearance, and out of all proportion to the change in urine flow, this exaltation being transient in the sense that the urea/creatinine clearance ratio may return to "normal" before the peak of diuresis is reached. The result obtained in experiment 72B followed the administration of 10 cc. of water per kgm. *per os*, which led to an immediate but small rise in urine flow, which then returned quickly to its previous level. The urea/creatinine clearance ratio increased abruptly, maintained the abnormally high level for two periods and then fell as the urine flow decreased. Fifty cubic centimeters of water per kilogram were then administered *per os*, and resulted in a second abrupt increase in the urea/creatinine clearance ratio, the increase being maintained as the urine flow was rising, but returning after the peak of diuresis to the normal level. Under certain conditions (exp. 74B) the exaltation of the urea clearance may persist as long as the urine flow continues to rise, but there

[8] We are convinced that these variations in the urea/creatinine clearance ratio are not due to errors introduced by the dead space of the pelvis and ureters of the kidney. Our experiments have been so designed that the plasma level of creatinine has remained very constant throughout all periods of observation (see tables 1 and 2); and dead space, though it might affect the absolute values of both clearances, would in no way affect the urea/creatinine clearance ratio. The extent to which dead space would alter the absolute value of a clearance will be determined by the rate of acceleration of urine flow, the initial and final U/P ratios, and the duration of the period of observation. The exact evaluation of the dead space error would be possible from our creatinine data if this clearance were constant throughout the experiment, but unfortunately this is not the case. The creatinine clearance is usually depressed to some extent in the control periods (at low urine flows) and increases after hydration of the animal (figs. 4 and 5); there is also an inconstant increase in creatinine clearance following the administration of water, as mentioned above. But the fact that occasional, rapid increases in urine flow, starting at low values, may not be accompanied by significant changes in the creatinine clearance suggests that the dead space in the normal kidney of an upright dog is relatively small. The apparent maximum dead space, calculated from the experiment detailed in table 2, is 0.5 cc.

is no relationship between the maximal exaltation and the peak of diuresis.

Eighteen experiments of this general type were performed, and in only the one case mentioned (26B) did the urea/creatinine clearance ratio fail to rise excessively on a rising urine flow. In general the rise in this ratio is most marked when the initial urine flow is low; we have been unable to obtain it when the initial urine flow was 2 cc. or higher (see table 2). It can be repeatedly evoked in a continuous experiment, as is shown by experiment 71B.

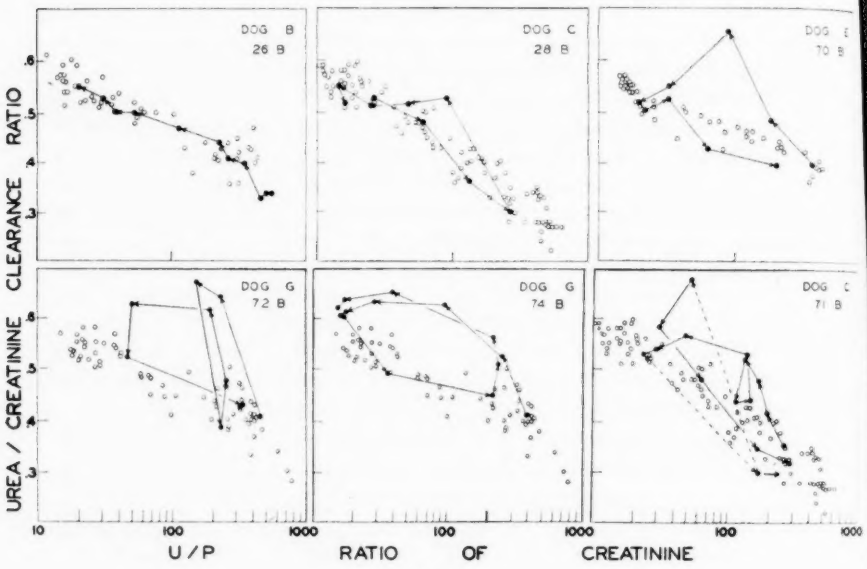


Fig. 7

It seemed possible that the phenomenon of the exaltation of the urea clearance on rising urine flow might be due to sudden hydration of cells of the renal tubules. This point was examined in six experiments, three of which are shown in figure 8. In these experiments control periods were taken at low urine flows; varying amounts (6 to 100 cc.) of 50 per cent sucrose were then injected intravenously, which caused an acute diuresis. The urea clearance rose relative to the creatinine clearance, just as though the urine flow had been increased by the oral administration of water. We must suppose that the renal tubules are at least relatively impermeable to sucrose, and that the presence of this substance in the interstitial fluid and the tubular urine would tend to dehydrate the tubule cells, in so far as it has any effect at all on local water equilibrium. In other experiments, in

which there was no exaltation of the urea clearance, the urine flow was maintained at a low level by the subcutaneous injection of 1 cc. of pitressin in a 20 kgm. dog, while rapid hydration of the body was effected by the

Fig. 7. The exaltation of the urea clearance during water diuresis.

The circles represent observations on stationary or falling urine flows, as recorded in figure 6. The dots represent observations during water diuresis. The arrows indicate the sequence of the experimental periods.

Experiment 26B, dog B (low protein diet). Water was withheld for 24 hours previous to the experiment. Following three control periods water was administered in quantities of 7 cc. per kilo *per os* every half-hour. The urine flow started to rise in 2.5 hours and during the next four hours maintained a steady increase. The logarithmic relationship between the urea/creatinine clearance ratio and U/P ratio of creatinine was exactly retraced. The periods were approximately 30 minutes in duration. The creatinine clearance was constant throughout.

Experiment 28B, dog C (low protein diet). Water *ad lib* previous to experiment. One hour before the beginning of period one, 15 cc. of water per kilo were administered by mouth. At the end of the fourth and sixth periods, there were given 7.5 and 25 cc. of water per kilo, respectively. The urea/creatinine clearance ratios observed on the rising urine flow approximated the "normal" values, except for the first period of diuresis, in which there was a slight exaltation. The periods in this experiment varied from 30 to 40 minutes. The creatinine clearance, after remaining fairly constant throughout the first seven periods (57.9–63.0), increased to 71.7 and 67 during the last two periods.

Experiment 70B, dog E (low protein diet). Water *ad lib* prior to the experiment. Following a control period, 12.5 cc. per kilo of water *per os* were given and observations were continued during the next three hours. The periods were approximately 20 minutes in duration. This is the most characteristic response obtained. The creatinine clearance rose steadily throughout the first five periods (50.8–58) and gradually returned to the control level.

Experiment 72B, dog G (high protein diet). Water *ad lib* prior to the experiment. This experiment is described in detail in the text. The periods were approximately 20 minutes in duration. The creatinine clearance rose during the fourth period (68.7–78.0), reached a peak of 86 in the eighth period and returned to 76 during the last two.

Experiment 74B, dog G (high protein diet). Water *ad lib* prior to experiment. Forty cubic centimeters of water per kilo were given after the first period and 20 cc. per kilo after the tenth period. The periods were approximately 20 minutes in duration. No significant change occurred in the creatinine clearance.

Experiment 71B, dog C (high protein diet). Water *ad lib* prior to the experiment. At the beginning of the first period 12 cc. of water per kilo were administered and the sixth period was followed by 25 cc. of water per kilo. The marked fall in the eleventh period in both urine flow and the urea/creatinine clearance ratio was spontaneous, as was the following rise (see dotted lines). The urine flows in these three periods were 3.75, 0.571 and 1.57. The arrow indicates that the true mean U/P ratio of creatinine in the eleventh period was much lower than was actually observed; while most of the water was excreted at a high urine flow, the urea in large part must have been excreted at a urine flow significantly below 0.571 for this figure to be the mean value for water excretion during this period. The periods were approximately 20 minutes in duration. The creatinine clearance rose irregularly throughout the first eleven periods (60–93) and then decreased to 83.

administration of 50 cc. of water per kgm. These conditions should effect the hydration of the renal tubules locally, as well as the body cells generally. That pitressin does not specifically prevent the exaltation of the urea clearance is shown in experiment 85B. In this experiment 1.0 cc. of pitressin was given subcutaneously and, after a control period, diuresis was induced by the intravenous administration of sucrose (5 grams in 10 cc. of solution). We infer from these experiments that neither pitressin nor the hydration of the body as a whole, or of the renal cells in particular, is the primary

TABLE 2

A typical experiment (no. 78B, dog E, low protein diet) to illustrate the exaltation of the urea clearance on rising urine flows caused by the administration of water

Note that when the urine flow was caused to increase from a high value no exaltation was found. This latter acceleration was slight when compared to that occurring from low urine flows. One hundred and 50 mgm. per kilogram of creatine were given at zero hour and 3:30. Fifty and 30 cc. of water per kilogram were administered by stomach tube at 1:13 and 4:35.

PERIOD	TIME FROM ZERO HOUR	DURATION OF PERIOD	URINE	PLASMA CONCENTRATION		U/P RATIO		CLEARANCE		$\frac{\text{UREA}}{\text{CREATININE}}$ CLEARANCE RATIO
				Urea	Creatinine	Urea	Creatinine	Urea	Creatinine	
		minutes	*cc./minute*	*mgm. per cent*	*mgm. per cent*			*cc./minute*	*cc./minute*	
1	40–1:13	33	0.109	8.10	7.58	172.0	479.0	18.8	52.2	0.360
2	—1:39	26	0.154	8.65	8.43	154.0	326.0	23.7	50.2	0.472
3	—2:01	22	1.14	8.98	8.43	33.9	51.1	38.7	58.3	0.664
4	—2:27	26	3.23	9.27	8.50	9.09	17.6	29.4	56.9	0.516
5	—2:54	27	4.07	9.43	8.58	7.93	14.7	32.3	59.8	0.540
6	—3:24	30	3.10	9.38	7.79	9.49	18.9	29.4	58.6	0.502
7	—3:49	25	2.1	9.24		11.85		24.9		
8	—4:09	20	2.6	9.12	9.63	10.06	21.9	27.6	57.0	0.484
9	—4:35	26	2.31	8.95	10.3	11.3	24.5	26.1	56.6	0.461
10	—4:55	20	2.45	8.74	11.36	11.1	22.3	27.2	54.6	0.498
11	—5:15	20	3.05	8.60	11.58	9.82	18.3	29.9	55.8	0.536
12	—5:39	24	3.33	8.50	11.43	8.97	17.1	29.9	57.0	0.525
13	—6:06.5	27.5	3.27	8.40	10.82	9.57	17.5	31.8	57.2	0.556

factor concerned in this phenomenon. Rather it appears that the exaltation of the urea clearance is directly related to an abrupt change in the extent of water reabsorption, as reflected in the U/P ratio of creatinine, or to the rate of water excretion, considered mechanically.

Discussion. It is unnecessary to amplify the discussion of the experiments reported above except in respect to urea. It would appear that the concept of an augmentation limit relative to the excretion of urea in dogs is erroneous. The apparent existence of an augmentation limit in previous

data has arisen from two circumstances; first, the relationship between the urea clearance and urine flow (at constant and falling flows) varies quantitatively from one animal to another, and the massing of data from different animals, even though the data were collected under suitable conditions,

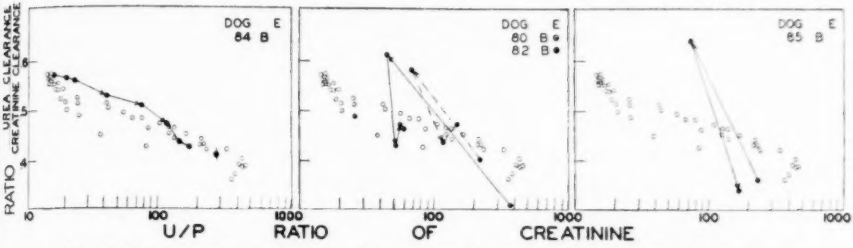


Fig. 8. Data showing the effect of sucrose solutions intravenously, with and without pitressin.

Experiment 84B, dog E (low protein diet). Water *ad lib* prior to experiment. Following one control period 25 grams of sucrose in 50 cc. of solution were administered intravenously. The urea and creatinine clearances were then determined during the following 4 hours. Periods were approximately 30 minutes in duration. The creatinine clearance fell following the injection of sucrose and then returned to its initial level in the third period, thereafter remaining constant (53.2–43.1–52.7 cc. per minute which was maintained.

Experiment 80B, dog E (low protein diet). Prior to the experiment water was withheld for 36 hours. Following one control period 10 grams of sucrose in 21 cc. of solution were given intravenously. Periods were approximately 20 minutes in duration. Creatinine clearances were 50.8, 41.1, 54.6, 57.8 and 58.3.

Experiment 82B, dog E (low protein diet). Water *ad lib* prior to the experiment. Following one control period 5 grams of sucrose in 10 cc. of solution were injected intravenously. Periods were approximately 20 minutes in duration. Creatinine clearances were 47.3, 49.7, 49.8 and 54.4.

Experiment 85B, dog E. Water *ad lib* prior to the experiment. One half hour before the beginning of the first period, 1 cc. of pitressin (10 pressor units, Parke, Davis & Co.) was given subcutaneously. Following the first period 3 grams of sucrose in 6 cc. of solution were administered intravenously. Periods were approximately 20 minutes in duration. Creatinine clearance was 43.4, 46.9, 46.4. Note the depression in urea/creatinine clearance ratio in the control period and following the exaltation.

It should be noted that the maximum urea/creatinine clearance ratio observed in these experiments is no indication of the true maximum actually reached; the period showing exaltation is usually preceded and followed by periods in which this ratio is relatively low, and this fact tends to obscure the real magnitude of the rise.

would tend to obscure the true relationship. Secondly, the phenomenon of the exaltation of the urea clearance on rising urine flows, which appears only under these specific conditions, tends to give maximum urea clearances at intermediate or low urine flows, and thus tends to obscure the true downward drift of the urea clearance, as described in this paper.

The exaltation of urea clearance on rising urine flows is undoubtedly the same phenomenon as was observed by Bourquin and Laughton (1925). Although we cannot explain its mechanism we believe that the phenomenon is a physiological one—i.e., having eliminated the mechanical error of tubular, pelvic and ureteral dead space, we must assign its genesis to the cells of the renal tubules. It can be evoked when the flow of urine is increased in the normal diuretic response to water, as well as when diuresis is evoked osmotically by intravenous sucrose, and it apparently does not depend on the state of hydration of the body. The facts that it is transient, lasting not longer than one hour, that the maximal urea clearance does not coincide with the maximal diuresis, and that it may be absent if the urine flow is increased slowly, indicate that it is the acceleration of the rate of urine flow that evokes the phenomenon rather than some change in the renal tubules, physiologically associated with decreased water reabsorption.

The most important aspect of our data concerns the question of why urea is reabsorbed in the mammalian kidney. (We set aside here as a feature secondary to the main problem, the excessive reduction of the urea clearance occurring at very low urine flows in dehydrated animals.) It was suggested by Rehberg (1926) and Holten and Rehberg (1931) that the progressive depression of the urea clearance as the urine flow was lowered was due to passive diffusion, in consequence of the increase in the concentration gradient across the tubules resulting from water reabsorption. But, in the light of our present results, it is impossible to accept this simple explanation. In a simple physical system, the change in concentration of any solute, due to diffusion across a permeable membrane, will be exponentially related to time. Given a constant creatinine clearance in the kidney, and assuming that the volume of the tubule distal to the site of water reabsorption remains constant, the U/P ratio of creatinine can be taken as a term directly proportional to the time during which a unit volume of urine remains in contact with a unit area of the tubular surface. Therefore, substituting $k \times U/P$ of creatinine for t in the ordinary diffusion equation, and letting $U\text{-}P$ represent the diffusion gradient, this equation becomes:

$$K = \frac{1}{U/P} \cdot \log \frac{1}{1 - \frac{a}{1 - \frac{P}{U}} \cdot \left(1 - \frac{Vu}{Vb}\right)}$$

Here U and P refer to the concentration of creatinine in bladder urine and plasma, a is 1—urea/creatinine clearance ratio, and Vu and Vb, respectively, the volumes of the urine and blood opposed to each other across the permeable surface.

In the equation as written above, it is supposed that the entire reab-

sorption of urea occurs distal to the site of reabsorption of water, and is dependent on the diffusion gradient of urea, essentially as suggested by Rehberg. But in addition the equation does, and must, take into account the time during which the concentrated urine is in contact with the tubule cells (a factor proportional, as we remarked above, to the creatinine U/P ratio), for at all urine flows this time element is a major factor determining the relative amount of urea reabsorbed.

But even in this amended form the diffusion hypothesis can not be made to fit the experimental data at both high and low urine flows. If the deficit in the urea clearance at a creatinine U/P ratio of 10 (which deficit amounts to 40 per cent) is due to a process of passive diffusion which is uniform with the process occurring at low urine flows, then at a creatinine U/P ratio of 100 the time of contact is by prediction long enough for diffusion to effect the approximately equal distribution of urea between blood and urine. A further increase in the creatinine U/P ratio should therefore not be accompanied by a further deficit in the urea clearance. The fact that there is no evident tendency for the urea/creatinine clearance ratio to level off before, or to become constant at creatinine U/P ratios of 100 would indicate that the simple diffusion hypothesis as stated above is inadequate. Apparently factors other than the diffusion gradient of urea and the time of contact are involved.

It is improbable that the failure of the simple diffusion hypothesis is due to our initial assumption of a constant tubular volume, *per se*, for any change in *Vu* of whatever magnitude would not obviate the predicted equilibrium between tubular urine and plasma at a creatinine U/P ratio of 100. But a change in *Vu* would affect both the extent of the permeable surface and the time of contact, and might affect K by altering the specific permeability of the tubule cells. It seems unlikely that the first two factors, which oppose one another, would be significant, though changes in the specific permeability of the tubule cells might well play an important rôle.

But as a second and perhaps more attractive hypothesis, it may be supposed that the reabsorption of urea involves two or more processes, or mechanisms, which are physiologically independent of each other. The deficit in the urea clearance observed at high urine flows may in part be of different genesis than the progressively increasing deficit occasioned by raising the creatinine U/P ratio above 10 (i.e., lowering the urine flow below 6 to 8 cc. per minute), which latter phenomenon has the appearance of increasing diffusion due to prolongation of the time of contact. But at the present moment no choice between these alternatives can be made on the available evidence. We feel that it is better to forego speculation in the hope that a new experimental approach will resolve some of the questions involved.

It is obviously impossible to interpret the excessive depression of the

urea clearance in dehydrated animals, or the exaltation on rising urine flows, until more is known about the fundamental factors involved in the major reabsorptive process or processes.

I wish to express my appreciation of the interest and critical assistance of Professor Homer W. Smith throughout this work.

SUMMARY

1. The urea clearance in relation to urine flow has been examined under a variety of conditions in six dogs (824 clearance periods), the simultaneous creatinine clearance being used in the majority of the periods in five dogs to measure the rate of glomerular filtration.

2. The rate of glomerular filtration is essentially constant and unrelated to the rate of urine flow in the ordinary experimental range of the latter. It may, however, be depressed by dehydration, or elevated by the administration of large doses of water.

3. At the highest urine flow obtainable (creatinine U/P ratio = 10) about 40 per cent of the urea filtered is reabsorbed. As the urine flow decreases an increased fraction of the filtered urea is reabsorbed; the increase in the reabsorbed fraction being approximately related to the logarithm of the creatinine U/P ratio.

4. Diuresis following a low urine flow is accompanied by a marked, transient exaltation of the urea clearance relative to the creatinine clearance, which may disappear before the peak of diuresis is reached. This exaltation is evoked by osmotic diuresis in a normal or pituitrinized dog, as well as by water diuresis in the normal.

5. The concept of an augmentation limit with its corollary of standard and maximum clearances does not appear to be applicable to the excretion of urea in the dog.

6. It is pointed out that a simple diffusion hypothesis, positing that urea escapes from the tubule distal to the point of reabsorption of water, and in consequence of the concentration gradient created by this reabsorption, is inadequate to explain the observed relationships between the deficit in the urea clearance and the rate of urine formation.

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THE EFFECT OF ACUTE HEMORRHAGE ON THE EMPTYING TIME OF THE STOMACH[1]

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In 1933 Van Liere, Crisler and Robinson reported that anoxemia produced a prolongation of gastric emptying time in the dog. In 1936 the senior author with the aid of Lough and Sleeth using man as a subject obtained comparable results with those found in the dog. It seemed logical to assume that if anoxemia produced a delay in gastric emptying time, anemia would probably do likewise, as the latter condition produces anoxia.

In order to test the validity of this hypothesis, however, it was deemed worth while to study the effect of hemorrhage on the gastric emptying time. This study seemed important as man often suffers more or less severe losses of blood from various causes.

METHODS. The experiments were performed on normal human beings and dogs.

A. *Man.* The normal gastric emptying time was ascertained fluoroscopically on four healthy male medical students. The standard meal was given at 8:30 a.m. These subjects had had no food since the evening before. The meal was prepared as follows: 15 grams of Quaker Farina were added to 350 cc. of water; this mixture was cooked until the total volume was 200 cc. One gram of salt was added for flavor. After the meal had cooled, 50 grams of barium sulphate were added so that the gastric contents could be seen with the fluoroscope. A number of control observations were made on each individual. The average of these figures was used for the norm.

The effect of hemorrhage was now studied. One-tenth of the calculated blood volume was withdrawn from the subject. It was assumed for the purpose of these experiments that the blood constituted 7 per cent of the total body weight. Using this figure, a man who weighed approximately 150 pounds (68 K.) was bled 500 cc. After the blood had been withdrawn, the subject ate the standard meal and the gastric emptying time was again determined. Attention was paid to all details necessary for carefully controlled work.

[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

B. *Dogs.* The normal emptying time of the stomach was determined in two vigorous dogs. The standard meal used in the dog was different from that used in man, as in the past 5 years a great deal of data on the normal gastric emptying time in the dog has been obtained using this particular meal (Van Liere, Crisler and Wiles). It was made up of the following ingredients: 40 grams of hamburger steak, 10 grams of dried ground bread and 50 cc. of milk; to this were added 15 grams of barium sulphate. A number of normal observations on the gastric emptying time were made and the average of these figures was used for the control. As was done in man, one-tenth of the calculated blood volume was withdrawn. The dogs were lightly anesthetized while the blood was being withdrawn. About an hour after they had been bled they were allowed to eat the standard meal and the gastric emptying time was again determined.

After the animals had entirely recovered from the hemorrhage (several weeks later) the effect of ether anesthesia on the gastric emptying time was determined. They were lightly anesthetized for the same length of time as they had been when the blood was withdrawn. About an hour after they had recovered from the anesthetic they were given the standard meal. It was found that the gastric emptying time was prolonged 38.5 per cent in the first animal and 29.6 per cent in the other. The next day, however, the gastric emptying time was normal in both animals.

Results. The accompanying tables show the results obtained.

The data in tables 1 and 2 clearly show that hemorrhage has a distinct effect on the gastric emptying time. The average delay in the gastric emptying time in the 4 human subjects who ate the standard meal immediately after the blood had been withdrawn was 41 per cent. The meal eaten 24 hours later showed a delay in three subjects from 15 to 20 per cent. One man showed a normal emptying time at the end of 24 hours. For all practical purposes gastric motility in man returned to normal in all the subjects after 48 hours.

Dogs showed a greater prolongation of the gastric emptying time than did man. On this account it was necessary to delay giving the second standard meal for 24 hours. In the dog it was found that gastric motility returned to normal on the fourth day instead of the third day as in man.

Discussion. In evaluating the data obtained in these experiments on man the psychic factor must be considered. It is admitted that it is impossible to rule this out. It can be said, however, that the subjects used in this work were young men who were familiar with the minor operative procedures employed and they looked upon the experiments as an ordinary routine procedure and an interesting experience. Two of the subjects who volunteered for this work had been used the previous year in studying the effects of low oxygen tensions on the gastric emptying time. There was far more danger in this latter experiment than in those reported in this

paper. The fact that gastric evacuation was still delayed on the second day showed that the psychic factor probably was not a very important one.

Hemorrhage caused the gastric emptying time of the dogs to be greatly prolonged. As mentioned earlier, the ether anesthesia was responsible for some delay for control experiments showed that ether anesthesia was capable of causing a delay in the gastric emptying time of about 35 per cent. This figure, however, is relatively unimportant as the gastric emptying time was delayed over 300 per cent after the blood had been withdrawn.

TABLE 1

The effect of hemorrhage on the gastric emptying time in man

NUMBER	NORMAL		IMMEDIATELY		24 HOURS LATER		48 HOURS LATER	
	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
1	2.0	100	3.0	150	2.0	100	2.0	100
2	1.7	100	2.5	147	2.0	117	1.5	88
3	2.6	100	3.3	127	3.0	115	2.7	104
4	2.5	100	3.5	140	3.0	120	2.7	108
Average..	2.2	100	3.1	141	2.5	113	2.2	100

TABLE 2

The effect of hemorrhage on the gastric emptying time in the dog

NUMBER	NORMAL		IMMEDIATELY		48 HOURS LATER		72 HOURS LATER		96 HOURS LATER	
	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent	Hours	Per cent
1	6.4	100	21.5	336	14.0	219	8.0	125	6.5	103
2	6.7	100	20.7	309	13.5	202	7.5	112	6.7	100
Average..	6.55	100	21.1	322	13.7	210	7.7	118	6.6	101

Ether, furthermore, could not account for the tremendous delay in the emptying time observed after 48 hours.

The standard meal given the dog contained a good deal of fat and protein; this meal could not be compared to the carbohydrate meal which was given the human subjects. Another factor might be mentioned, that is, gastric secretion is probably diminished after a hemorrhage and this may have delayed the stomach emptying time. At the present time this phase is being studied.

The results reported in this paper correlate very well with the results obtained when working with low oxygen tensions, which work has already been mentioned. It is felt that the same mechanism is involved, namely,

the loss of blood caused an anoxia. The mechanism of the prolongation of gastric emptying time produced by anoxemia has been shown (Crisler, Van Liere and Wiles) to be on a vagospastic pylorospastic basis. After a certain threshold is passed, however, further delay is produced by a definite loss of gastric motility.

It is of interest to note that human subject number 2 showed a decrease of 12 per cent in the gastric emptying time on the third day. Comparable results were obtained in certain subjects under very moderate degrees of anoxemia. It would seem that mild degrees of anoxemia can actually stimulate gastric motility.

The results indicate that patients who have suffered a considerable loss of blood should be given food which is easily digested and which will leave the stomach rapidly. Mention must be made of the fact that a loss of blood of 500 cc. in man cannot be regarded as a grave hemorrhage. Blood donors are often called upon to give this amount and more. The subjects, moreover, reported in this paper were not exposed to shock. It is quite possible that the delay in the gastric emptying time would be much greater if the subject suffered from shock and hemorrhage both.

SUMMARY AND CONCLUSIONS

The normal gastric emptying time was determined fluoroscopically in four healthy male subjects. After one-tenth of their calculated blood volume was withdrawn (about 500 cc. in a 68 K. man), the gastric emptying time was prolonged an average of 41 per cent. In no case was it less than 25 per cent. Twenty-four hours after the hemorrhage three of the four men still showed a delay from 15 to 20 per cent. One subject showed no delay 24 hours after the blood had been withdrawn. At the end of 48 hours the stomach had apparently regained its normal motility in all the subjects.

Observations were also made on two dogs which had had one-tenth of their calculated blood volume withdrawn. The dogs too showed a distinct prolongation in gastric emptying time confirming the results found in man.

The authors wish to express their sincere thanks to Dr. C. B. Pride for withdrawing the blood from the human subjects.

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TOTAL PLASMAPHERESIS

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Since Abel, Rowntree and Turner (1914) introduced the method of plasmapheresis the technic has been widely used to study the physiological consequences of partial plasma removal. Usually the washed cells have been re-suspended in colloid-free Ringer-Locke solutions, and re-injected. Using this method Smith, Belt and Whipple (1920), Whipple, Smith and Belt (1920) and Belt, Smith and Whipple (1920) always observed fatal shock in dogs when the plasma proteins were reduced below 1 per cent. They concluded that "blood serum proteins are stabilizing or protective factors" and that "physiological perfusion of organs is very difficult, and slight modification of the blood plasma may have a profound effect upon body cells." Drinker (1927) observed capillary dilatation and edema in the web of the frog perfused with Ringer solution containing 3 per cent gum acacia. Upon the addition of as little as 15 per cent horse serum these phenomena disappeared. He concluded that "serum possesses a peculiar power of restraining capillary dilatation and . . . leakage." Krogh (1929) similarly concludes that "there is evidently something in . . . serum, apart from its colloidal properties, which is essential to keep the capillaries of the frog's web in a normal state of permeability. . . . The power may be inherent in the serum proteins or it may be a special hormone." Drinker and Field (1933) state that probably "in acute plasmapheresis capillaries all over the body experience change, becoming temporarily more permeable to protein."

It is, however, certain that many cells and tissues in the body are not thus dependent upon the presence of proteins in their fluid environment. Such fluids as aqueous humor, glomerular filtrate, and cerebro-spinal fluid are normally nearly, or quite, protein free. Tissue fluid is believed by many to have a very low protein content (Krogh, Landis and Turner, 1932; Landis, Jonas, Angevine and Erb, 1932; Peters, 1935). However others consider that higher values, approximating those in lymph, are normally present in tissue fluid (Drinker and Field, 1933).

Our own interest in the problem of the functional significance of the

plasma proteins was first aroused in connection with our work with hemoglobin-Ringer (Amberson, Flexner et al., 1934), in the course of which we observed that all but the last traces of the normal blood could be washed out and replaced by this solution without evidence of shock. It was obvious that, under our experimental conditions, the normal plasma proteins must be reduced to the vanishing point, and we stated that "either hemoglobin in solution is able effectively to replace the proteins . . . in their control of normal function, or the ill effects ascribed to their diminution or absence have been incorrectly interpreted, and caused more by changes in the physical properties of the blood than by any specific chemical influence." Since hemoglobin is also a protein we have now completely removed the blood plasma by using solutions containing gum acacia, a colloidal polysaccharide.

METHOD. From three or four animals (cats or dogs) we collect sufficient blood to furnish cells for the experiment. The serum is removed from the defibrinated blood, and the cells are three times washed through Ringer-Locke. A total plasmapheresis requires 300 to 400 cc. of solution per kilo of body weight. The washed cells are mixed with Ringer-Locke containing 6 per cent gum, in the proportion of 30 to 40 per cent cells to 70 to 60 per cent gum-saline. In adding the salts we omit the $CaCl_2$ since the acacia contains enough Ca to balance the solution. We use 200 mgm. per cent glucose and 20 mgm. per cent $NaHCO_3$.

The mixture of cells with gum-saline must not be made until the very moment of the infusion. Gum causes a very rapid sedimentation of the red cells (Lucia and Brown, 1934). If the mixture is allowed to stand some of the cells form clumps which cannot be broken up even by vigorous shaking. These act as emboli and may give rise to a variety of central nervous disorders.

The blood is removed under a light ether anesthesia. The carotid artery is brought through the skin by way of a small incision, and cannulated. We find it convenient to use a water-jacketed cannula whose temperature is held at about 45°C. by passing hot water through the outer jacket. The animal is first bled through a side tube and the blood volume is then restored by infusing an equal volume of red-cell-gum-saline through the main tube of the cannula. In cats we remove and inject 50 cc. at a time; in dogs we use 100 cc. Infusion is backward toward the heart under pressure.

In dogs, after plasmapheresis, further blood samples are readily secured by venous puncture; we therefore close the neck wound at once. Several weeks later it is possible to cannulate the same artery again and repeat the operation. In cats blood sampling is more difficult. We usually leave a small glass cannula in place in the carotid and find it possible to keep the wound reasonably clean by the application of bandages soaked in Dakin's fluid, or other antiseptic solution. Samples are drawn through this

cannula for the first three days. After this time it is usually necessary to recannulate farther down on the same vessel. We have not attempted to keep cats for extended periods, killing them after six to nine days. Dogs, however, are easy to keep indefinitely, even after two successive plasma removals.

Plasma proteins have been determined by the Kjeldahl method after fractionation as prescribed by Peters and Van Slyke (1932). In a few experiments acacia has been determined. Values accurate to 10 per cent are secured by removing the proteins by trichloracetic acid precipitation, followed by alcoholic precipitation of the acacia. In the presence of 70 per cent alcohol acacia precipitates slowly and may later be collected in a Gooch crucible and weighed. Such alcoholic precipitation was suggested (but apparently not used) by Keith, Power and Wakefield (1935).

Results. It is not possible, by our method, to reduce the protein concentration in the circulating blood to absolute zero. All of the original blood-plasma may be washed away, but it is impossible to remove all of the tissue fluid and lymph, so that proteins drain back into the blood stream from extra-vascular regions. By repeated bleedings and infusions the plasma proteins may be reduced to a level of 0.05 to 0.15 per cent; in addition there is always present a trace of vegetable protein from the gum (0.1 per cent in the 6 per cent solution).

The recovery of the animal can hardly be distinguished from that of a normal one after a similar period of etherization. We have had animals recover so quickly that they took food within two hours. Edema is never seen in cats, and only rarely in dogs. In the latter it never appears in the first day, when the proteins are at their lowest value. The internal organs of animals killed within an hour or two after the plasma removal show no pathological condition. When edema does sometimes appear in dogs, in the second, third or fourth day, it is a transient phenomenon, localized in the neck region.

A maintained blood pressure furnishes the most striking evidence that a very low plasma protein concentration is not necessarily accompanied by shock or capillary dilatation. In cats the blood pressure usually drops somewhat during the course of the plasmapheresis, but it recovers quickly, and may even rise somewhat above the original normal value. In dogs the blood pressure maintains itself with remarkable constancy, falling, of course, with each bleeding, but rising back to normal as the blood volume is restored by the next infusion. An example of such constancy in the blood pressure is shown in figure 1. In this experiment the total plasma protein fell from an original value of 6.29 per cent to 0.09 per cent at the time of the last graphic record. The terminal blood pressure is almost exactly the same as before the plasmapheresis. It is certain that no condition even remotely resembling shock could have been present at this time.

Rectal temperatures invariably decline during the course of the plasmapheresis, but the fall is not greater than that observed in normal animals after etherization for similar periods. Temperature recovery is prompt.

Rate of disappearance of gum acacia. When relatively small amounts are injected gum acacia is known to disappear fairly rapidly from the blood stream (Meek and Gasser, 1918; Huffman, 1929; Peoples and Phatak, 1935). After our massive injections acacia leaves the blood stream more slowly, possibly because the liver and other tissues, now known to act as storehouses for acacia (Andersch and Gibson, 1934), become completely filled. Thus in one typical experiment we found that, at the end of 22 hours, the acacia had fallen to 68 per cent of that originally present. At 44 hours it was 54 per cent; at 93 hours, 32 per cent; at 162 hours, 26 per cent; and at 334 hours, 4 per cent.

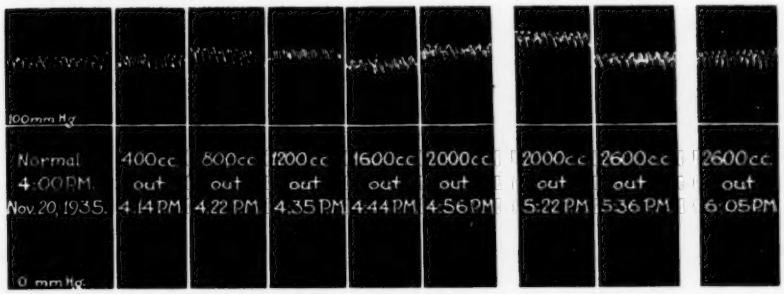

Fig. 1. Constancy of the blood pressure in a dog as its normal blood is removed and replaced by dog red-cell-gum-saline solution. In the course of the plasmapheresis the plasma proteins are reduced from 6.29 per cent to 0.06 per cent. After the second pause the proteins rise to 0.09 per cent. A trace of vegetable protein from the gum is also present.

Rate of replacement of the plasma proteins. In our preparations it is possible to follow the return of the plasma proteins from a nearly zero level. In figure 2 we show the rate of replacement in four dogs after total plasmapheresis. The various protein fractions are shown as well as the total. Replacement is complete in about 200 hours. The fibrinogen returns rapidly, whereas the albumin and globulin rise more slowly. Throughout the recovery the albumin-globulin ratio approximates that found in the original blood.

During this recovery the animals are fed, first milk on the first day, then bread and milk for several days, finally a mixed diet ad lib. We recognize the importance of diet in determining the rate of plasma protein replacement (Pommerenke, Slavin, Kariher and Whipple, 1935), but have not been immediately interested in studying this influence. Our diets have contained sufficient protein of high potency to give us a rapid rate of replacement. The data are submitted as evidence for the ability of the animals to reconstitute a normal blood after total plasmapheresis, rather than

as studies of the replacement rate per se. We believe that this method may furnish a new approach to the study of dietary factors in plasma protein regeneration.

We have kept some of our dogs for as long as three months after the operation. They are entirely normal in every respect. They also survive after a second complete removal of the blood plasma two or three weeks after the first plasmapheresis. The rate of replacement of the proteins, after a second removal, is identical with that observed after the first. We have seen no evidence of the effect described by Dick, Warweg, and Andersch (1935) who found that repeated injections of gum-saline acted drastically to reduce the plasma proteins. Our experimental conditions are rather different from theirs.

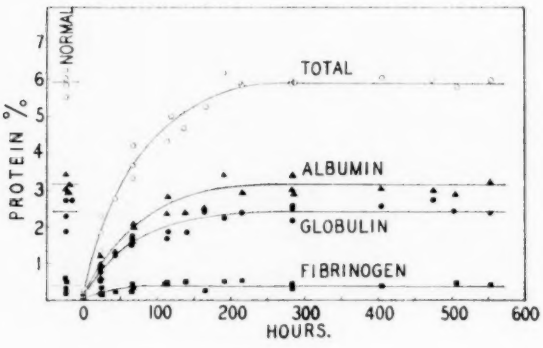

Fig. 2

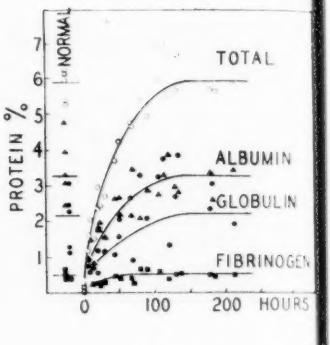

Fig. 3

Fig. 2. Replacement of plasma proteins in four dogs after total plasmapheresis.
Fig. 3. Replacement of plasma proteins in six cats after total plasmapheresis.

In figure 3 we show the rate of replacement of plasma proteins in six cats after total plasmapheresis. The rate is definitely more rapid than in dogs. Normal values are reached in about 125 hours. The albumin-globulin ratio is highly variable during the recovery period, but the total protein returns at practically the same rate in all animals.

Discussion. It is clear that the mammalian body can survive after the removal from the blood of all but the last traces of the plasma proteins. For short periods, at least, they may be replaced, not only by the protein, hemoglobin, as in our earlier experiments, but by the colloidal polysaccharide, gum acacia. Unfortunately for the strength of this demonstration a very low plasma protein cannot be maintained for long. There is evidence (Holman, Mahoney and Whipple, 1934, and others) that a plasma protein reserve exists in the mammalian body. There is also a considerable quantity of protein in tissue fluid and lymph, presumably separate from the true reserve. From several sources, therefore, protein

is quickly mobilized. Thus in five dogs (of another series than that given above) in which total protein was originally 5.77 per cent, on the average, the value fell to 0.08 per cent after total plasmapheresis, but rose to 1.01 per cent four hours later.

We consider that, in many plasmapheresis investigations, the observations have been vitiated by a failure to maintain an adequate oxygen supply to the various tissues of the body. Certainly the original method of Abel, Rowntree, and Turner (1914), in which the washed cells are returned to the body suspended in colloid-free Ringer-Locke, must inevitably lead to tissue edema and diminished blood volume, with partial asphyxiation as an immediate consequence. Lack of oxygen is known to exercise the most powerful influence upon capillary permeability (Landis, 1928). In the perfused mammalian heart Ort, Power and Markowitz (1931) and Ort and Markowitz (1931) have found that, in the absence of red cells, gum saline is unable to maintain the beat, with much gum leaving the blood stream and penetrating into the tissue, whereas, when sufficient cells are present, little or no gum leaves the vessels, and the heart beats for some hours. Since gum actually diminishes the oxygen capacity of the whole blood (Christie, Phatak and Olney, 1935) the danger of insufficient red cells, or of reduced blood volume, becomes even more evident.

From our observations we conclude that the walls of the blood vessels, and the tissue cells generally, are by no means so sensitive to low protein in the plasma as many workers have believed. The plasma proteins undoubtedly give certain essential physical properties to blood plasma, but other colloids, even colloidal polysaccharides, can take their place. It is still conceivable that there may be a capillary hormone controlling permeability, but these results make it highly improbable that the plasma proteins are responsible. We consider our results to support the view that these proteins have mainly a physical significance, giving a proper viscosity and colloidal osmotic pressure to the blood and thereby regulating its flow and volume.

SUMMARY

The blood plasma may be almost totally removed from the blood vessels of cats and dogs, without injury to the animals, if the normal blood is replaced by a solution of 6 per cent gum acacia in Ringer-Locke, in which are suspended washed red cells of the same species (30 to 40 per cent).

This substitution is done by alternate bleeding from, and injection into, the carotid artery, until a large volume of the solution, of the order of 300 to 400 cc. per kilo, has been passed through the body. The plasma proteins may thus be reduced to the very low level of 0.05 to 0.15 per cent.

No signs of shock, or of capillary dilatation and edema, appear after such total removal of the plasma. The blood pressure is maintained, and the animals recover quickly and may live indefinitely.

When an adequate diet is given the plasma proteins return to normal in about 200 hours in dogs, and 125 hours in cats.

It is concluded that the plasma proteins are not specifically necessary for the maintenance of normal function. Other colloids, even polysaccharides such as gum acacia, may be substituted for them, if these are able to maintain blood volume, and if sufficient oxygen is present in the solution. The major function of the plasma proteins is physical, in regulating blood flow and volume, by maintaining the viscosity and colloidal osmotic pressure of the plasma.

It is a pleasure to acknowledge the support given to this work by the American Philosophical Society, from the Penrose Fund, and by the Council on Pharmacy and Chemistry of the American Medical Association. We are also indebted to the Eli Lilly Company, of Indianapolis, for supplying the acacia.

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THE ACTIVITY OF THE CARDIAC SYMPATHETIC CENTERS

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The development of methods for recording the nerve impulses going to and from the centers provides an effective means for studying the activity of the sympathetic nervous system (Adrian, Bronk and Phillips, 1932). Such a direct analysis of the behavior of the sympathetic nerve cells is especially valuable because it is difficult to determine their functional characteristics from the response of the organs which they supply. The inertia of these effectors is so great that the activity produced by the individual nerve impulses is masked or obscured, a situation which is quite different from that in the somatic system where a study of the contraction in striated muscle has given much information regarding the properties of the individual motor nerve cells.

In the present investigation we have undertaken to determine the character of the sympathetic nerve discharge to the heart. The purpose of the experiments has been to add to our knowledge concerning the activity of the sympathetic nervous system and to give a clearer understanding of the nature of cardiac control.

METHODS AND PROCEDURES. Most of the experiments were performed on cats under light nembutal anesthesia but in a few cases decerebrate animals were employed without significantly different results. In every case the animal was kept in a humid chamber at a temperature of about 34°C. The chest wall was removed from the first to the sixth ribs, after which respiration was maintained by means of a pump. It was then possible to reach the long cardiac-sympathetic nerves which run from the stellate ganglia to the inferior cardiac plexus and contain both chronotropic and inotropic fibers. One of these nerves was dissected free from the surrounding tissues and transected some distance from the heart. The proximal portion was then slung onto silver-silver-chloride brush electrodes leading to a direct-coupled or resistance-capacity-coupled amplifier and

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Matthews oscillograph. In this way the efferent sympathetic impulses in the cardiac nerves were recorded.

RESULTS. *The tonic discharge.* Records of the sympathetic discharge to the heart are shown in figure 1. In all our experiments on 46 animals we have found such continuous activity in the cardiac sympathetic nerves, provided the animal was in reasonably good condition. This statement is not to be construed, however, as meaning that the degree of that activity is constant. It is indeed increased by asphyxia, decreased by overventilation or a rise in blood pressure, and largely modified by numerous other factors. But in general, under the conditions of our experiments in which the blood pressure and depth of respiration were permitted to vary within wide limits, there was a persistent discharge of impulses. This is positive evidence, then, in support of the view held by Hunt (1899) and others that the heart is under a continual influence of sympathetic impulses.

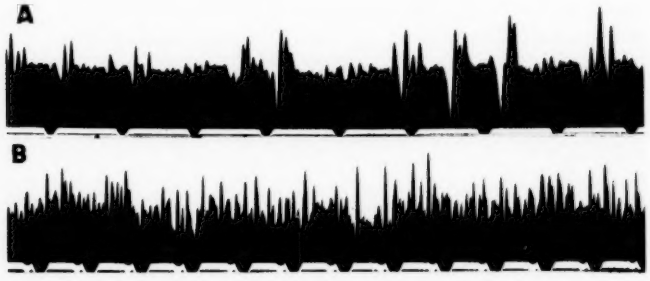

Fig. 1. Records showing ungrouped "tonic" discharge of impulses in a sympathetic cardiac nerve from the stellate ganglion. A, in relatively few fibers; B, in the whole nerve. Time marker in this and subsequent records gives $\frac{1}{5}$ second intervals.

The fiber pathways. The impulses shown in figure 1 and elsewhere in this paper have been recorded in postganglionic fibers for they are abolished by painting the stellate ganglion with nicotine. The pathways of the preganglionic impulses by which they are initiated have been determined by cutting successively the first to the fifth thoracic rami. When the first or second thoracic ramus is severed there is little decrease in the magnitude of the postganglionic discharge. Section of either the third or fourth ramus, on the other hand, produces a very considerable decrease, and when they are both cut practically all the impulses are eliminated. Our experiments show therefore that most of the sympathetic impulses going to the heart from the stellate ganglion enter by way of the third and fourth thoracic rami.

The course of many of the postganglionic fibers is in one or more nerves running from each ganglion to the cardiac plexus. A considerable number

of the fibers, however, go directly from the ganglia to the vagi and run with those nerves toward the heart so that it is always possible to observe a discharge of sympathetic impulses in the vagus nerves.

It is of course not possible in our experiments to restrict the fibers under observation to those concerned with the control of the rate and force of the cardiac beat. But anatomical evidence indicates that the great majority of the efferent fibers in these nerves going to the cardiac plexus from the stellate ganglia have that function. It is fair to assume therefore that our results give typical evidence concerning the activity of the sympathetic nerve cells regulating the heart.

The source of the potentials. The type of action potential with which we are dealing is illustrated in figure 2A. A maximum electric stimulus was applied to a postganglionic nerve from the stellate ganglion and the response recorded after an interval which indicates that the impulses are

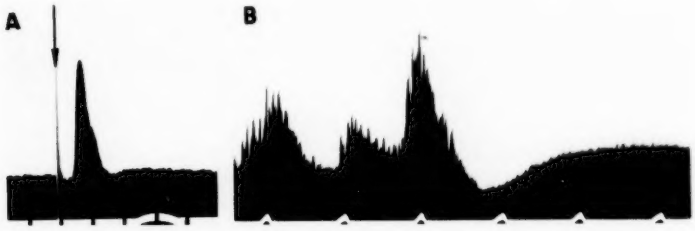

Fig. 2. A. Action potential in a postganglionic nerve from the stellate ganglion elicited by a single shock to nerve (indicated by arrow). Time: 0.025 second. B. Positive after potential revealed when the normal efferent discharge in the postganglionic fibers was suddenly inhibited reflexly by a rise in blood pressure in aorta and carotid sinuses produced by clamping the descending aorta.

transmitted with a velocity which varies from 0.6 to 1.5 m. per second in different fibers. They are characterized by large positive after potentials which become especially apparent at the end of a period of sustained stimulation or whenever the normal efferent discharge is suddenly inhibited reflexly as in figure 2B.

The individual potential pulses in such efferent discharges as are shown in figure 1 are of considerable magnitude and may be as great as 50 microvolts. They are therefore too large to be the action potentials in single postganglionic fibers. In discussing similar large potential pulses in the hypogastric and cervical sympathetic nerves Adrian, Bronk and Phillips (1932) suggested that they are produced by many postganglionic fibers functioning as a unit and in fairly close synchronism. This is because one preganglionic fiber activates many postganglionic fibers (Billingsley and Ranson, 1918). Thus the ganglion functions as a natural amplifier.

The random and irregular character of the discharge in figure 1 indicates that we are dealing with more than one such group of postganglionic fibers innervated by a single preganglionic fiber. We have frequently tried to observe the impulses in a single unit by cutting and splitting the trunk but the small size of the fibers and the fragile character of the nerve make this a difficult task. One can more readily record the impulses in a single preganglionic fiber, and that is just as useful in analyzing the activity of the individual sympathetic motor nerve cell. In such single preganglionic fibers we have observed impulse frequencies as low as one or two per second and seldom higher than ten or twenty a second. This is in marked contrast with the activity of somatic motor nerve cells from which the rate of impulse discharge may be five or ten times as great (Adrian and Bronk, 1928). Such differences are interesting in view of the slower characteristics and greater inertia of the organs supplied by the sympathetic nervous system. Mammalian skeletal muscle fibers require 30 to 350 impulses a second in order that they may develop their maximum tension (Cooper and Eccles, 1930), whereas sympathetic stimulation of 10 to 15 a second produces maximum cardiac acceleration (Bronk and Pumphrey, 1935). Bozler (1936) has also found that low frequencies of sympathetic stimulation (2 3 a second for the frog) are sufficient to maintain the blood vessels in a state of maximal constriction, and only when the frequency of sympathetic stimulation is low are dorsal root impulses effective in modifying the degree of contraction.

Grouped impulses. One of the most striking characteristics of the nervous discharge in the cardiac sympathetic fibers is the frequent grouping of impulses to form waves which may be several hundred microvolts in amplitude. Because of their size and their form they almost certainly represent the activity in many more postganglionic fibers than are supplied by a single preganglionic fiber. These waves are therefore not due to the type of ganglionic grouping referred to above. It might still be argued, however, that they are the result of some property of the ganglion whereby the activity of the various units is synchronized. Such a possibility is indeed suggested by Tower's (1933) interesting observation of wave-like groups of impulses in sympathetic fibers from the isolated viscera of a frog. She tentatively proposes that "it is not beyond possibility that the cellular plexi of the viscera or ganglia contain something analogous to a synchronizing mechanism."

It is more probable however that these volleys of impulses which we are considering may be due to a more or less synchronous rise and fall in the level of excitation of large numbers of sympathetic motor cells in the cardiac centers. That this is the correct explanation is shown by the fact that similarly grouped impulses are observed in the preganglionic nerves.

Such synchronized firing of the cells in the sympathetic centers is further

illustrated by an experiment which was designed to observe the relation between the activity on the two sides of the body. A nerve from the right stellate ganglion was placed on electrodes leading to one amplifier and oscillograph; a nerve from the left stellate ganglion was placed on electrodes leading to a second amplifier and oscillograph. In this way simultaneous records were made of the impulses from the ganglia on the two sides. A

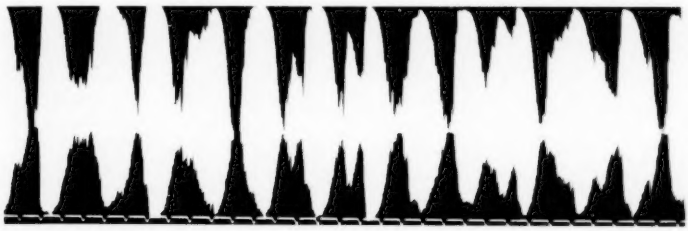

Fig. 3. Grouped impulses in right and left sympathetic cardiac nerves recorded simultaneously with two recording systems, to show synchronization of activity on the two sides.

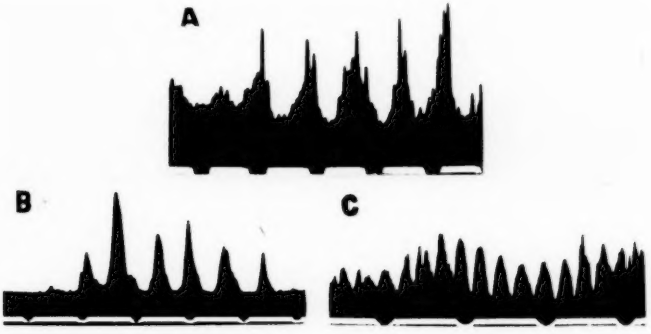

Fig. 4. Efferent sympathetic cardiac impulses grouped into regularly recurring waves. A. Record from relatively few fibers. Frequency of waves, 6 per second. B. More fibers in action, lower amplification. Frequency 7 per second. C. Frequency 20 per second.

typical record is shown in figure 3, where it will be seen that the groups of impulses from the two ganglia are synchronous. Because this rhythmic activity is bilaterally synchronous we must conclude that the groups of motor cells on the two sides are closely connected, so that they readily interact and modify each the other's activity, or are subject to a common driving influence which causes their rhythmic excitation. This is reminiscent of the findings of Gasser and Newcomer (1921) on the discharge of

motor impulses in the phrenic nerves. Recording simultaneously the impulses in the nerves on both sides they found rapidly recurring waves of activity within each respiratory cycle which occurred simultaneously in the two nerves. From such experiments they decided that "the intervals at which impulses are discharged from the cord into the nerve from the two sides are controlled by the same common point."

The groups of impulses in the cardiac nerves are sometimes discharged at irregular intervals, or at other times with marked regularity as shown by figure 4. In A the frequency of the waves is about 6, in B about 7 and in C they occur 20 times a second. These are three rhythms commonly observed in the sympathetic system; the form of the waves indicates that

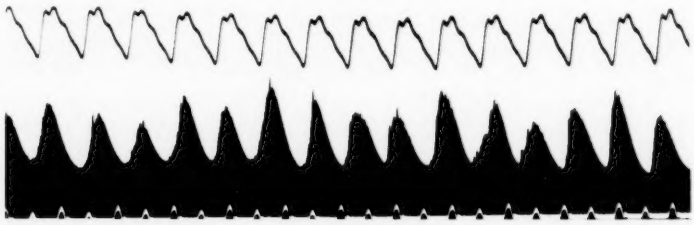

Fig. 5. Grouped discharge with the frequency of the cardiac cycle. Carotid pulse in the uppermost record.

Fig. 6. Periodic inhibition of the sympathetic discharge with each inflation of the lungs. Inflation is shown as a downward movement of the middle record (plethysmograph).

there is a periodic increase and then decrease in the number of motor nerve cells in action. This cyclical fluctuation in the activity of a large group of cells in the central nervous system, occasionally at a rate corresponding to that of the Berger rhythm, is of especial interest at the present time when considerable attention is being paid to rhythmic processes in the brain.

Such regularly recurring waves as are described in the previous paragraph do not often persist continuously for more than a few seconds at a time. A rhythm appears for a short interval, then disappears or becomes obscured by a more irregular activity, and reappears again. Two other types of rhythmically grouped discharges of lower frequency are more persistent, and the mechanism of their production is more susceptible to analysis. They are discussed in the next section.

Cardiac and respiratory groupings. Although the rhythmic volleys of impulses we have been considering are related to no other obvious rhythm of the organism we have often observed groups of impulses in phase with the heart beat or respiration. This periodic activity synchronous with the cardiac or respiratory cycles has already been found in sympathetic nerves supplying constrictor impulses to blood vessels (Adrian, Bronk and Phillips, 1932).

An instance in which the impulses are grouped into large waves, occurring with the frequency of the heart, is shown in figure 5 where synchronous with each cardiac cycle there is a large volley of efferent sympathetic impulses. The activity of the motor nerve cells is in some way inhibited or stimulated synchronous with the pulse, so that there is a rhythmic beating of the centers. The mechanism will be discussed later.

Less frequently we have observed a grouping of the impulses with respiration as shown in figure 6, the discharge occurring during expiration.

Fig. 7. Volleys of sympathetic impulses synchronous with the pulse. As pressure falls cardiac rhythm disappears and discharge becomes more continuous. Uppermost record: pressure in carotid artery.

In many animals both the cardiac and respiratory groupings are present at the same time, as in the figure presented.

It is apparent that those groups of motor nerve cells which control the rate and force of the cardiac contraction possess no one characteristic rhythm. The cells show a marked tendency to work in synchronism with a resulting grouped discharge; but the rhythm with which these volleys occur is highly variable for the sympathetic centers appear to be extremely labile and subject to various influences. Certain of these can be described.

Afferent control of sympathetic rhythms. There are two fairly obvious explanations of the bursts of sympathetic impulses which occur with the frequency of the pulse. A. The periodic pulsation of blood pressure within the central nervous system might act as a mechanical stimulus to the motor nerve cells causing their periodic excitation. B. Bronk and Kaltreider (1931), and Bronk and Stella (1932), have shown that there is a volley of afferent impulses discharged from the receptors in the arch of the aorta and the carotid sinus with each systolic rise in pressure. These rhythmically

recurring groups of impulses, playing upon the sympathetic centers, may cause them to function with a corresponding rhythm.

Our experiments show the latter to be the most important, if not the sole cause, of the cardiac grouping for section of the carotid sinus and aortic nerves usually abolishes such synchronized activity in the sympathetic nerves. A frequent observation which gives further support to this view is illustrated in figure 7. Previous to the beginning of the record the blood pressure in the upper part of the animal had been elevated by clamping the descending aorta; it will be observed that as the record begins the sympathetic cells discharge a volley of impulses with each cardiac cycle. When the pressure falls there is more continuous activity and no simple grouping of the impulses synchronous with the pulse. The lower pressure is a less effective stimulus of the receptors in the carotid sinus and aorta, and the consequent reduction in number of afferent impulses makes less effective their influence on the sympathetic centers. It seems probable, therefore, that when the excitation of the cells in the cardiac sympathetic centers rises and falls with the frequency of the heart the variation in activity is somehow due to the pulsatile volleys of afferent impulses from the walls of the blood vessels.

In rare cases there is a suggestion of a cardiac rhythm in the sympathetic discharge after both carotid sinus and aortic nerves are cut. In such cases there is the possibility that impulses from blood vessels which travel over other afferent pathways may be the responsible agents. Thus Gammon and Bronk (1935) have reported impulses from Pacinian corpuscles in the mesentery which are sometimes in volleys synchronous with the pulse, and recently such grouped afferent impulses have been observed in fibers passing through the stellate ganglion (Bronk and Larrabee, 1935).

The cessation of sympathetic impulses during inspiration has been described earlier in the paper. Such rhythmic activity appears to be due to periodic inhibition of the centers by afferent impulses from stretch receptors in the lungs, for the rhythm disappears after section of the vagi. This control of the cardiac sympathetic centers is further illustrated by experiments in which the lungs are distended to varying degrees and so maintained. On distention there is a complete inhibition of the sympathetic discharge for a period of time which depends upon the degree of inflation, and subsequently there is a gradual escape from the inhibition. Similar results are obtained by electrical stimulation of the central ends of the cut vagi.

Such a relation between the respiratory cycle and the activity of the cardiac sympathetic centers has been observed in fourteen animals; in only one have we found a discharge of impulses during inspiration and in that instance the rhythm persisted after section of the vagi. This latter observation recalls the experiments of Adrian, Bronk and Phillips (1932)

in which there was occasionally a respiratory grouping in the cervical sympathetic and hypogastric nerves, the discharge occurring during inspiration. Because the influence of respiration on sympathetic activity was greater after section of the vagi and because in the curari-immobilized animal there were bursts of sympathetic impulses synchronous with the motor discharges in the phrenic nerves it was concluded that the respiratory grouping "was due to the direct action of the respiratory center on the vaso-motor center." Our present experiments indicate that the cardiac sympathetic center may likewise be directy excited by the respiratory center but is more often under the inhibitory influence of afferent impulses from the lungs or, more often still, is free from any apparent nervous connection with respiratory activity.

Rhythmic driving of the centers. The preceding experiments indicate the importance of afferent impulses from the viscera in regulating the rhythmic

Fig. 8. Rhythmic waves of activity (grouped impulses), recorded in sympathetic cardiac nerve and reflexly driven by stimulating central end of aortic nerve at times indicated by arrows. Each wave is associated with the previous stimulus (interval between stimulus and the associated wave 0.3 sec.)

activity of the cardiac sympathetic centers. Such afferent determination of the efferent rhythm is well illustrated by the following observations.

During the course of an experiment in which we were stimulating electrically the central end of an aortic nerve we found at high stimulus frequencies inhibition of the centers and subsequent escape. But as the rate of stimulation was lowered the centers began to discharge rhythmically and with the frequency of the stimulus. This is shown in figure 8. When the frequency of afferent excitation was lower than a certain critical level the centers failed to follow, and the discharge again became random and irregular. We have repeated this on many different animals, stimulating either the aortic or carotid sinus nerves, and we frequently find that the cells comprising the cardiac sympathetic centers can be driven rhythmically at rates varying from about 2 to 15 per second. Apparently the time constants of the cells are such that they are unable to respond with synchronous and grouped discharges above and below these frequencies.

It is not improbable that the genesis of such artificially induced rhythms is similar to that of the grouped discharges synchronous with the pulse.

In both cases the mechanism would appear to be an inhibition of the sympathetic activity by the volley of afferent impulses, followed by release, and again inhibition by the next afferent volley. Thus a systolic rise in pressure initiates in the carotid sinuses and aorta bursts of afferent impulses which, arriving at the centers inhibit their activity. The discharge begins again during the latter part of diastole as in figure 5, reaches its maximum during the next systole and is again inhibited by the afferent impulses accompanying that systole. The phase of the cardiac cycle during which the impulses are observed in the sympathetic nerve will of course depend upon the conduction times for the afferent and efferent impulses and the latent period of the centers.

The sequence of events in figure 8 is probably to be accounted for in a similar manner but certain observations indicate that the complete explanation of the phenomenon may involve additional considerations. For instance, in two experiments the volleys of sympathetic impulses persisted for several seconds after the cessation of the stimuli and at the same regular frequency, a persistence which was much longer than could be accounted for by a lag or latency of the centers (only 0.3 sec. at the beginning of the stimulus). Only gradually did the centers lose the rhythm which had previously been imposed upon them by the volleys of afferent impulses. We must conclude that there are some obscure factors in the mechanism whereby the activity of the sympathetic motor cells is set into resonance with the afferent impulses.

Discussion. The importance of the sympathetic nerve supply to the heart has been debated frequently and some authors have doubted whether the accelerator and augmentor fibers are normally in action. The present experiments give definite evidence as to the actual state of activity in those fibers and show that they conduct a fairly continuous series of impulses to the heart. They thus support the view of Hunt (1899) and others that the heart is under a continual sympathetic influence. It is the opinion of such writers that the efficacy of vagal impulses is largely determined by the accelerator background existing at any moment; the greater the sympathetic activity, the greater the effect produced by a given variation in vagal discharge. If such be the rôle of the cardiac sympathetic impulses, effective control of the heart would depend in considerable measure upon a ready modification of the sympathetic discharge by impulses reporting the condition of the viscera. Such a lability of the cardiac sympathetic centers under the influence of afferent impulses has been amply revealed by our experiments and is in agreement with the original observations of Hering (1894) and of Hooker (1907) which established the reflex control of the cardiac accelerator nerves.

The pronounced grouping of the efferent impulses which results from the rhythmically synchronized discharge of the motor nerve cells appears as

one of the most striking and characteristic properties of the sympathetic system. This is not without analogy in somatic nerves although there the frequency of the grouping is usually quite different. For instance, Dittler and Garten (1912) and Gasser and Newcomer (1921) have shown that in the electric response of the whole phrenic nerve there are oscillations at the rate of about 70 a second. Adrian and Bronk (1928) subsequently found that when the individual motor nerve cells are discharging at a low frequency there is no synchronization of the discharge from the several cells; not until the frequency of impulses rises to 60–70 a second does any appreciable synchronization develop. Thus a smooth contraction is maintained for if there were synchronized groups of impulses of a low frequency they would cause a gross tremor of the muscle. Here in the sympathetic nerves, however, we find the frequency of the impulse volleys varying from one every 3 or 4 seconds to about 20 per second. It is of interest to consider their effect upon cardiac activity.

The nature of the cardiac musculature and its relation to the extrinsic nerves are such that it is probably of little significance whether the impulses in the several fibers arrive synchronously or out of phase with one another. But the relative effects of volleys of impulses recurring at frequent or infrequent intervals is less obvious. In preliminary experiments (Bronk and Pumphrey, 1935) we have imitated these efferent volleys of various frequencies by electrically stimulating the peripheral end of a sympathetic cardiac nerve. When short bursts of impulses are delivered more frequently than every five seconds no rhythmic variation in the heart rate is observed. If the groups of stimuli are applied at longer intervals than that, the heart rate shows fluctuations synchronous with the periods of stimulation. From this it would appear that the only type of efferent sympathetic rhythm which could be reflected in the cardiac rate is the respiratory grouping, and that only when the rate of respiration is fairly slow.

This raises the question as to whether the periodic inhibition of the cardiac sympathetic discharge is in part responsible for sinus arrhythmia. It is generally believed (cf. Samaan, 1935) that the arrhythmia is due to variations in vagal activity for it is said to be abolished by atropine or by section of the vagi. On the other hand, our experiments show that the sympathetic discharge to the heart does frequently vary with respiration and that these variations are capable of producing changes in the heart rate.

SUMMARY

The activity of the cardiac sympathetic centers has been investigated by recording the action potentials in the cardiac nerves from the stellate ganglia of the cat.

There is a fairly continuous discharge of impulses which exert a "tonic" augmentor and accelerator influence upon the heart. This discharge is however largely modified by changes in the chemical composition of the blood and by afferent impulses.

The principal pathways of the impulses from the cord to the stellate ganglion are the third and fourth and to a lesser extent the second and fifth thoracic rami.

The impulse frequency from the individual sympathetic motor nerve cells seldom exceeds ten or fifteen a second, and is usually considerably less. This contrasts with the much higher frequency of discharge from somatic motor nerve cells.

The potential pulses in the postganglionic nerves are of considerable magnitude because of the grouping of impulses which results from the innervation of many postganglionic fibers by a single preganglionic fiber.

There are also much larger potential waves caused by the synchronous activity in very many nerve fibers. It is shown that this is due to the coordinated and rhythmic discharge from large numbers of nerve cells in the centers. This activity is bilaterally synchronous.

The grouped activity is of four types. The volleys may come at irregular intervals or at other times periodically with frequencies varying from 5 to 20 a second but unrelated to any other obvious rhythm of the organism. Or, on the other hand, the bursts of impulses may be synchronous with the pulse or the respiratory cycle.

The latter two forms of rhythmic cellular activity are largely due to afferent impulses from the viscera: bursts of impulses from the blood vessels initiated by the systolic rise in pressure or the impulses from distention receptors in the lungs.

An example of the marked effect of such afferent impulses upon the activity of the sympathetic centers is found in the observation that it is possible to drive those centers by repetitive stimulation of the central ends of the carotid sinus or aortic nerves, thus causing the motor nerve cells to discharge periodically with the frequency of the afferent impulses. This can be done within a limited range of stimulus frequencies.

The characteristically grouped discharges from the cardiac sympathetic centers cause periodic variations in heart rate only if the bursts of efferent impulses are separated by some seconds. This is due to the inertia of the effector mechanism.

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A POSSIBLE RÔLE OF THE EOSINOPHIL LEUCOCYTES IN THE ENDOCRINE COMPLEX OF THE FEMALE RAT[1]

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The experimental production of pseudopregnancy in white rats has been accomplished in two general ways: a, single stimulation of the cervix of the uterus, this, by initiating events leading to follicular inhibition and luteal activity, producing the typical early gestational picture in the uterus; b, repeated hormone administration, effecting the ovarian and uterine changes presumably by a more direct action.

Goat anti-rat thymus serum, non-specific and generally hematotoxic (Goldman and Kraatz, unpublished), injected intraperitoneally into 14 adult virgin female rats in doses of $\frac{1}{4}$ to $\frac{1}{2}$ cc. daily, resulted in 10 pseudopregnancies. The uteri of three of these pseudopregnant females were traumatized by the insertion of threads and responded with formation of macroscopic placentomata. All rats receiving doses of $\frac{1}{2}$ cc. and the smaller rats receiving $\frac{1}{4}$ cc. doses became pseudopregnant after having one or two normal cycles; while daily injections of 1 cc. of normal goat serum continued for three weeks did not affect the cycles.

The possibility that the gonadotropic hormone content of the immune serum was responsible for the effects described is doubtful since it was found that the serum had no influence on the ovaries or the maturing of young females. Furthermore, single intraperitoneal injections in 47 animals of $1\frac{1}{2}$ to 3 cc., given at prooestrus or early oestrus, resulted in 27 pseudopregnancies, nine being verified by securing positive decidual reactions. The failures may be attributed to the fact that this is apparently a "threshold reaction." Successive bleedings of the goat produced sera of varying titer and, where $1\frac{1}{2}$ cc. of a given lot would fail to evoke pseudopregnancy, 2 cc. of the same lot would succeed. The pseudopregnancies uninterrupted by autopsy ranged from 12 to 17 days in length with an average of 13.81 days. Neither normal goat serum nor goat anti-rat liver serum in single injections of 3 cc. affected the cycles. Occasionally, slight weight losses were noted following injections, the maximum being 4.9 per cent of body

[1] This paper is a thesis submitted by C. P. Kraatz to the faculty of the Graduate School of the University of Cincinnati in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoölogy, June, 1936.

weight in a rat injected daily, but recovery of initial weight was always completed after five days from the first injection and the animals gained from that point.

Similar doses of the anti-thymus serum in late oestrus or postoestrus lengthened the following dioestrous period to 3 or 4 days, while single injections during dioestrus produced a continued full oestrus of 3 or more days in 8 out of 14 animals. The ovaries of 2 of these when sectioned showed follicular cysts.

Thus, an anti-thymus serum, primarily affecting blood cells in the adult rat, was found to produce a heavily luteinized ovary by a single injection during prooestrus, and, in some cases, a cystic ovary by a single injection in dioestrus. It appeared probable that the prooestrous injections initiated a chain of events comparable to those producing normal pseudopregnancy.

The most constant effect of serum treatment was eosinophilia. Animals injected daily were found to have eosinophil leucocytes in the circulation during pseudopregnancy ranging from 6.5 to 11.5 per cent of the total white count, while controls receiving larger amounts of normal serum for the same length of time had 3 per cent or less. Single injections resulted in a slight eosinophilia within 24 hours, the conditions becoming pronounced in 48 to 72 hours and persisting for at least 4 days (table 1 and fig. 1).

The only generally accepted belief regarding the eosinophil leucocytes is their proliferative response to foreign proteins in the circulation. Daily leucocyte counts were made on female rats of various ages and stages of sexual activity. Differential counts of 500 white cells each (1000 in immature animals) were made in a longitudinal direction on Wright-stained smears. The product of the eosinophil percentage and the white cell count for that day resulted in a figure representing the number of eosinophil leucocytes per cubic millimeter of blood. Averages of these figures, based on differential counts of 127,500 cells, are used in table 1 and figure 1.

Only the counts for regular 4-day oestrous cycles are included in the figures for normal adult females in table 1. Additional counts of slightly aberrant cycles showed the lowest eosinophil number sometimes before prooestrus in the case of an abbreviated oestrus, or after prooestrus in the case of lengthened oestrus, but a rise always occurred following oestrus Individual animals maintained levels of eosinophil fluctuation relatively constant in successive cycles, but differing from the levels of other individuals. In general, the level rises with increase in age. Thus, the rats in the pseudopregnancy determinations, being young, showed lower levels in the normal cycles than the average.

Excepting the constant oestrous condition, the significant differences in the eosinophil number under various conditions parallel the probable

TABLE 1

CONDITION	SMEAR (VAGINAL)	EOSINOPHIL PER CENT	EOSINOPHILS PER C.MM.
Normal cycles (21 cycles in 15 rats)	Prooestrus	1.705	257 ±31.8
	Oestrus	2.015	307
	Postoestrus	2.5	432 ±28.2
	Dioestrus	1.88	305
Normal pseudopregnancy with preceding cycle (7 rats—see Note)	Prooestrus	0.95	125
	Oestrus	1.34	206
	Postoestrus	1.6	233
	Dioestrus	1.64	270
	Prooestrus (Sterile copulation)	1.5	188
	Oestrus	2.26	373
	Pseudopregnancy (2)	2.34	346
	Pseudopregnancy (3)	2.16	457
	Pseudopregnancy (4)	1.7	285
	Pseudopregnancy (5)	2.4	306
Serum pseudopregnancy with preceding cycle (4 rats—see Note)	Prooestrus	0.9	127
	Oestrus	1.73	266
	Postoestrus	1.8	423
	Dioestrus	1.86	352
	Prooestrus (Injection)	1.5	197
	Oestrus	1.775	217
	Pseudopregnancy (2)	3.55	755
	Pseudopregnancy (3)	4.0	841
	Pseudopregnancy (4)	2.7	822
	Pseudopregnancy (5)	3.3	786
Immature females (10)	None	0.59	59 ± 6.6
Adults with cystic ovaries (3 rats—7 days each)	Oestrus (constant)	4.41	666 ±44.4
50-day old littermates			
2 mature—5 days each	Cycles	1.16	240 ±26.6
2 immature—5 days each	None (closed)	0.18	45 ± 9.7

Normal cycles:	Difference between prooestrus and postoestrus	175 ±45.1
Normal pseudopregnancy:	Average of 5 days before stimulation	200 ±15.3
	Average of 5 days after stimulation	347 ±17.6
	Difference	147 ±23.3
Serum pseudopregnancy:	Average of 5 days before injection	305 ±25.4
	Average of 5 days after injection	705 ±21
	Difference	400 ±33.0

Note: In the pseudopregnancy determinations, the animals being young show, in general, lower levels of eosinophil fluctuation in their normal cycles than those in the averages for all ages given above.

variations in luteinizing hormone liberated from the anterior lobe of the pituitary gland under those conditions: i.e., low in immature animals, with a sudden rise at maturity; a rise during oestrus, contributing during this rise to the process of ovulation as suggested by Hisaw, Fevold, Foster and Hellbaum (1934); a rise during pseudopregnancy resulting in the formation of functional corpora lutea. Regarding constant oestrus, divergent opinions exist. Parabiosis experiments lead DuShane, Levine, Pfeiffer and Witschi (1935) to believe that the condition is due to lack of luteinizing hormone. On the other hand, Smith and Engle (1934) find ovarian cysts resulting from implantations of anterior lobe containing probably both follicular stimulating and luteinizing hormones, while injections of castrate

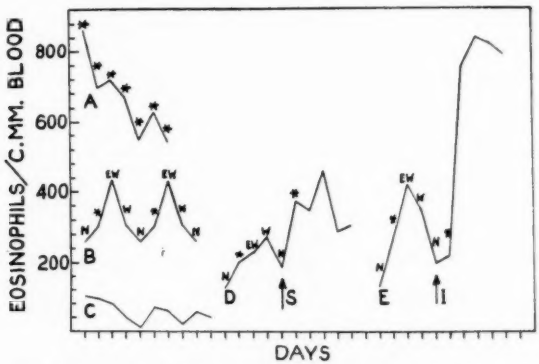

Fig. 1. Graph of figures in table 1, showing daily variations in concentration of eosinophil leucocytes under differing conditions. *A*, rats in constant oestrus. *B*, rats experiencing normal cycles. *C*, immature rats. *D*, pseudopregnancy induced by sterile copulation at (arrow) *S*. *E*, pseudopregnancy induced by serum injection at (arrow) *I*. Stages of cycle indicated by symbols: *N*—prooestrus; *—oestrus; *EW*—postoestrus; *W*—dioestrus.

urine containing only the follicular stimulating factor do not produce this effect. A high, relatively static balance of hormonal influence on the ovary as the causative factor in continued oestrus is indicated by the results of both groups. In the present experiments, the production of pseudopregnancy and cystic ovaries by a common treatment and the high eosinophil number in constant oestrus subscribe to this concept.

The observations above led to attempts to assay the eosinophil leucocytes of the female rat qualitatively for luteinizing hormone. Isolation of these cells has never been accomplished, but white cell suspensions of relatively high and low eosinophil content were compared in respect to their power to augment the action of follicular stimulating hormone in immature female rats. Suspensions of high eosinophil concentration were obtained from the

citrated blood of pregnant, serum-injected or constant-oestrous rats by repeated centrifuging and washing three times with physiological saline solution. Low concentrations were similarly obtained from non-pregnant and saline-injected adults. Greater eosinophil concentration was sometimes obtained by the use of hypotonic (0.75 per cent) saline in washing, while hypertonic (0.9 per cent) solution tended to have an opposite effect.

The follicular stimulating hormone used was a 60 per cent alcohol-soluble fraction of an aqueous pyridine extract of acetone-dried pig anterior lobe, prepared after the method of Fevold et al. (1933).

Subcutaneous injections of follicular stimulating hormone-plus-white cells were made twice daily into 22-day old rats for 3 days, the animals being autopsied on the fifth day. Ovaries were dissected free and weighed. There was considerable variability in the response of different litters to the control doses of like amounts of hormone, but, within each litter, the results were fairly constant. $ZnSO_4$, augmentative in action (Maxwell,

TABLE 2

HORMONE PLUS	ANIMALS	A	B	C	PER CENT AUGMENTATION	
					Range	Average
			cc.			
1. Over 1 million E.	11	50.85	0.177	2.154 ± .223	47–246	148.2 ±16.1
2. Under 1 million E.	9	33.2	0.167	0.513 ± .053	0–63	39.6 ± 5.46
Difference				1.641 ± .23		108.6 ±17.0

A. Average of millions of white cells received by each animal.

B. Average volume of blood elements (packed by centrifuging) received by each animal.

C. Average of millions of eosinophil cells received by each animal.

1934), injected in doses of 10 mg. in combination with follicular stimulating hormone into 4 rats from 3 different litters, although resulting in ovaries which ranged from 66 to 118 mgm. in weight, showed fairly uniform augmentation percentages of 433, 464, 500 and 503 over littermate control ovaries. Therefore the per cent of increase in ovarian weight over littermate hormone-injected controls rather than gross ovarian weight has been used in table 2. The 12 controls used had a range of 12.4 to 22.6 mgm. in ovarian weight with an average of 18.02.

The response is apparently proportional to the number of eosinophil leucocytes injected. Group 1, receiving on the average 319 per cent more eosinophils than group 2, demonstrated a 274 per cent greater ovarian response, while receiving 53 per cent more white blood cells and 6 per cent more total blood cellular material. The small difference in number of white cells injected can hardly be responsible for the increased augmentation in group 1, since ovarian response does not increase proportionally as

the amount of a non-hormonal substance administered is increased, e.g., casein (Saunders and Cole, 1936). Evans, Simpson and Austin (1933) achieved augmentation with 3 cc. doses of rat blood serum, greater from non-pregnant than pregnant females. Eosinophilia, provoked by blood-group antagonisms, is indicated by these results, especially since pregnant blood is believed to be higher in luteinizing hormone content than non-pregnant. Blood-group reactions, too, are probably responsible for the wide ranges shown in table 2, but this effect should be common to both groups, 1 and 2. Therefore, the difference in response is probably due to a difference in content of luteinizing hormone.

A number of non-specific substances have displayed augmenting properties when administered to rats in conjunction with purified or unfractionated pituitary extracts. Extracts of male urine, milk, egg white, lemon, horse thyroid and beef liver (Hellbaum, 1936); various blood sera and urines (Evans et al., 1933); formed elements of beef blood (Casida, 1936); casein and egg albumen (Saunders and Cole, 1936); serum from non-pregnant mares (Cole and Hart, 1934); tannic acid (Fevold et al., 1933); and $ZnSO_4$ (Maxwell, 1934) have proven effective in evoking this phenomenon. The tentative explanation, a delayed absorption of follicular stimulating hormone, may account for a portion of the augmentation, but, in view of the huge increases observed in non-hypophysectomized animals in response to non-hormonal substances, these have probably initiated events resulting in a steady liberation of luteinizing hormone from the living pituitary.

As shown by Lane and Hisaw (1934), the pituitaries of immature rats can be provoked to liberate luteinizing hormone by uterine cervical stimulation and by administration of oestrin. It seems believable that the unifying mechanism of the action of various injected substances may be a stimulation to eosinophilia, these cells providing an increased amount of luteinizing hormone in the circulation. An eosinophilia, persisting for some time after treatment, is expected in the case of foreign protein injections (guinea pigs, Biggart, 1932). Cole, Guilbert and Goss (1932) found gonadotropic mare serum with a high protein content as effective in a single dose as in divided doses, suggesting again the initiation of some mechanism such as that proposed, rather than direct hormonal action. The failure of $ZnSO_4$ and casein in combination to act additively in augmentation (Saunders and Cole, 1936) points to the identity of method of their action. The common pharmacological action of $ZnSO_4$ and tannic acid in the precipitation of proteins hints at the same mechanism. Preliminary experiments in this laboratory indicate a rise in eosinophils after injections of $ZnSO_4$ either alone or in combination with follicular stimulating hormone into immature females, while injections of hormone alone tend to lower the eosinophil number.

In view of the evidence presented, it seems credible that the concentration of eosinophil leucocytes in the circulation of the female rat may be an indication of luteinizing activity; and further, that the cells themselves may find a rôle in the control and transport of the luteinizing hormone.

SUMMARY

1. Goat anti-rat thymus serum either by single or repeated intraperitoneal injections induced pseudopregnancy in adult female rats, with accompanying eosinophilia.

2. The concentration of eosinophil leucocytes in the circulation of female rats of various ages appeared to vary as the probable concentration of luteinizing hormone of the pituitary.

3. Assays of leucocyte suspensions for luteinizing hormone showed those of high eosinophil cell content to be more effective in augmenting the action of follicular stimulating hormone in immature females than those of low eosinophil content.

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A STUDY OF THE SPEED OF ABSORPTION FOLLOWING THE INGESTION OF GLUCOSE AND OF SUCROSE

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Several different methods have been utilized for the study of absorption of carbohydrates from the gastrointestinal tract. One of these is well represented by the work of Cori (1925) which utilizes the intact gastrointestinal tract. In this method, the test solution is introduced into the stomach of the fasting animal by stomach tube, is allowed to remain for varying periods of time, after which the animal is killed, and the contents of the gastrointestinal tract are analyzed.

Ravdin et al. (1933) studied absorption from a "Thiry loop," or segment of intestine, as modified by one of them (Johnston, 1932). Into a loop segment made in this manner, the fluid to be tested is introduced, is allowed to remain for a predetermined period, after which it is withdrawn for analysis.

It has long been obvious to workers in the field that, after carbohydrate ingestion, the rise in the blood sugar level and its subsequent fall must be dependent on the rate of absorption of the sugar from the intestine, on the one hand, and on the rate of loss of sugar from the blood by various physiological means, on the other. In this study we have utilized the difference in the speed of rise of the blood sugar curves from their basal levels, after ingestion of glucose and of sucrose, as being indicative of the relative rates of absorption of those two sugars.

EXPERIMENTAL. The experimental animals were picked at random and therefore varied greatly in breed, age, and size. However, all four of the dogs were males. Their diet consisted of bread, lung, and bone meal, supplemented with cod liver oil. They received approximately fifteen minutes' exercise a day. The animals were trained to lie quietly over a period of several hours. Before each experiment they received no food for about twenty hours.

After being brought from the cage for the experiment, the dogs always rested for a half-hour period before the basal sample of blood was procured. As soon as this sample (1) had been drawn, the test fluid (6 grams per kilo of the sugar to be tested, in about 60 per cent aqueous solution) was intro-

[1] Fellow, Corn Products Research Fund.

duced into the animal by stomach tube, whereupon the dog was returned to its resting position in which it remained until the end of the experiment. At exactly one-half hour and one hour after the animal received its sugar, samples 2 and 3 respectively were taken for analysis. The blood was analyzed by the Shaffer-Hartmann method as modified by Somogyi (1921–1933).

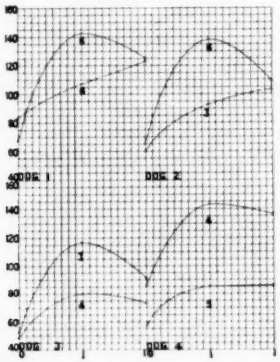

Fig. 1. Dog's blood sugar after ingestion of 6 grams per kilo of corn sugar and cane sugar. The curves are averages of the number of experiments indicated by the figures placed on each curve.

Results. The composite blood sugar curve of each animal, based upon blood sugar determinations following numerous glucose ingestions, rises more sharply and to a greater height during the first half-hour after glucose administration, than the comparable curve rises following sucrose ingestion.

Discussion. In contrast to the work of Ravdin et al. (1933), the present investigation was undertaken to study the relative speed of absorption of glucose[2] and sucrose in the normal, unanesthetized dog with its entire gastrointestinal tract functioning as a unit. Since it was desired to compare the action of two different sugars in the same dog, an acute experiment such as Cori performed would have been impractical. Also Cori's statistical treatment, made possible by the use of small animals (rats), would not have been feasible where dogs were the subjects. The method which was adopted for this study, the use of the rate of rise of the blood sugar curve as the indicator of the speed of absorption, allowed a large number of experiments to be performed on the same animal and utilized the normal gastrointestinal tract of the unanesthetized dog.

The choice of the amount of sugar, though quite arbitrary, was made to insure an excess of the sugar during the absorption period. The high concentration was deemed advisable, since the dogs which had a tendency

[2] "Dyno," a product of the Corn Products Company, was the source of glucose.

to regurgitate seemed better able to retain their sugar when the volume of the solution was limited. Cori (1925) had found that the absorption was not influenced by the absolute amount or by the concentration of the sugar present in the intestine.

When the stomach tube was administered to the animal, but no sugar was given, the rise in blood sugar was found to be negligible.

Although we agree with the position of Trimble et al. (1933), as based on their work and that of Johansson (1909), that there is probably "a lack of significance in its (the blood sugar curve's) return to normal as an indicator of the completion of the absorption process," our own results are not concerned with those portions of the sucrose and glucose curves in which there is a return to the basal blood sugar level, but rather with the rising portions of these curves, and their relative heights at the end of the first half-hour after administration of the test solution. During this early period that still elusive mechanism, which tends to throw the blood sugar curve back to normal before absorption has stopped or even slowed, can scarcely be responsible for the fact that the blood sugar curve rises more slowly after sucrose than after glucose ingestion, since the influence of this mechanism to throw the curve back to normal is much more marked in the case of glucose than it is in the case of sucrose. It would seem from the curve, then, that this mechanism has not become an important factor during the period to which we are confining our attention and from which we are drawing our conclusions.

It has been shown that a large percentage of carbohydrate entering the blood stream (by injection) disappears rapidly therefrom (Bang, 1913). However, Cori (1927–28) has demonstrated that this may be recovered from the tissues and that all sugars penetrate into the tissues at the same rate, i.e., the tissues are not selective in taking up different sugars as are the intestines. Therefore, while this fact would affect the use of the blood sugar level as an indicator of the absolute rate of absorption, it does not prevent the use of the blood sugar level as an indicator of which sugar is entering the blood stream more rapidly through the intestines.

SUMMARY

Numerous experiments have been carried out to determine the relative blood sugar level after glucose and after sucrose ingestion in the unoperated, unanesthetized dog. There appears to be a very definite tendency toward a higher blood sugar level after glucose than after sucrose ingestion. This difference is interpreted to be due, at least in part, to the faster rate of absorption of glucose than of sucrose from the gastrointestinal tract.

I wish to express my appreciation to Dr. A. J. Carlson for suggesting this problem and for his valuable advice throughout the course of the work

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ELECTRICAL STIMULATION OF THE INTERIOR OF THE CEREBELLUM IN THE DECEREBRATE CAT[1]

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Responses to faradic stimulation of the interior of the cerebellum of the monkey have been described by Magoun, Hare and Ranson (1935). The present paper is concerned with the reactions similarly obtained in normal and decerebrate cats.

Stimulation within the cerebellar nuclei and the adjacent fiber paths was accomplished with the aid of the Horsley-Clarke stereotaxic apparatus, the use of which has been described by Ranson (1934). As in the experiments on the monkeys, a needle-like bipolar electrode, the exposed tips of which were 1 mm. apart, was used to conduct the exciting current led off from the secondary coil of a Harvard inductorium. The primary coil, connected to a single dry cell providing a flow of 1.5 ampere when in circuit with the inductorium, was 9 cm. from the secondary. Stimuli were applied at millimeter intervals along the course of each puncture so that a thorough exploration was obtained in each case. The cerebellum was prepared for microscopic examination and the position of the tips of the electrodes during each stimulation was anatomically identified. Of the twelve decerebrate cats used, ten were stimulated with vertical electrodes; the other two decerebrate and all the normal cats were stimulated with horizontal electrodes. All of the cats were suspended by the frame of the stereotaxic instrument and by strings passing under the supraspinous ligaments, an arrangement which permitted free movements of all parts of the body.

The decerebrate cats were operated under ether anesthesia which was discontinued when the brain stem was severed so that during the period of stimulation the animal, though completely unconscious, was not subjected to the depressing effect of an anesthetic.

All the reactions to cerebellar stimulation present two definite phases: a first which is coincidental with the electrical stimulation, and a second, usually the contrary of the first, which appears as a rebound after the cessation of the stimulation and which continues for a time varying from a few seconds to five minutes or more. It became apparent during the course of the experiments that the majority of the cerebellar responses were readily

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divisible into two great groups; one consisting of the responses of the head, trunk, tail and all four extremities in a manner closely similar to the tegmental reaction (Ingram, Ranson, Hannett, Zeiss and Terwilliger, 1932), and another including the reactions of the ipsilateral forelimb not admissible to the first. That such separation of the responses is at least partly natural and not wholly artificial is evidenced by the localization of the first group near the midline in the vermal portion of the cerebellum and of the second group in the lateral parts of the vermis and the most medial parts of the interior of the cerebellar hemispheres.

Within the first category a very great number of the responses are in conformity with the following pattern: During stimulation there is a flexion of the ipsilateral limbs, extension of the contralateral, deviation of the head, trunk and tail with the concavity toward the side of stimulation. The rebound, lasting for several seconds to several minutes, makes its appearance only after the end of the electrical stimulation and consists of extension of the ipsilateral limbs, flexion of the contralateral and formation by the vertebral axis of an arc concave to the side opposite the point stimulated. A response fulfilling this pattern in every detail is illustrated in photographs G and H of figure 1. The pictures, all of decerebrated cats, are arranged in pairs representing the two phases of the reaction to stimulation of a single point within the cerebellum. The upper photograph of each pair was taken during the first phase, the lower during the rebound. The point stimulated to produce the postures shown in G and H was in the left side of the cerebellum. In photograph G, taken while the cerebellum was being stimulated, the head, and concavity of the trunk and tail are directed to the left; the left or ipsilateral limbs are powerfully flexed, and the right legs are extended. In the rebound, illustrated in photograph H, the animal assumed a posture the opposite of that obtaining during stimulation; the entire body axis is an arc concave to the right, the right limbs are flexed, and the left are in full extension.

A less vigorous response of the same pattern is illustrated in E and F of figure 1, but in this instance the electrodes were in the right side of the cerebellum. A still weaker reaction to faradization within the right side of the cerebellum is seen in C and D. Stimulation caused generalized inhibition or relaxation but the rebound posture was that of tonic extension of the right limbs, flexion of the left, and a slight concavity of the spinal column to the left. Stimulation of some points in the cerebellum produced such weak or attenuated reactions that only one member of the body would participate. Turning of the head to the right during stimulation when the electrodes were in the right side of the cerebellum is portrayed in photograph A; the rebound, in which the head is turned to the left, is shown in B.

On following the photographs in the sequence in which they have been described it is obvious that the reactions become progressively weaker and

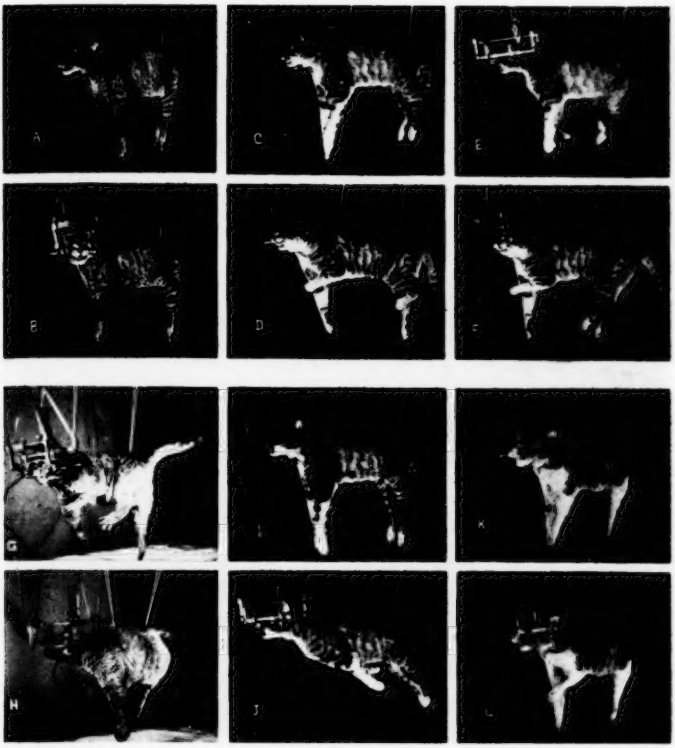

Fig. 1 contains six pairs of photographs portraying the responses of the decerebrated cat to cerebellar stimulation. Each pair represents the two phases of the response to stimulation of a single point within the interior of the cerebellum, the upper picture in each case showing the first phase which is coincidental with stimulation, and the lower one the second phase or rebound occurring after stimulation. All of the photographs except K and L show reactions obtained from the cerebellar vermis. Pictures A and B show a weak response, in which only the head is active, to a stimulus applied in the right side of the cerebellum. C and D also illustrate a response to stimulation in the right side of the cerebellum but in this case the limbs took part in the reaction. Photographs E and F present a still stronger reaction to a stimulus in the right side of the cerebellum. During the first phase the right limbs are slightly flexed, the left extended, and the vertebral column curved to the right. On the rebound the opposite prevails; the right limbs are extended, the left are flexed and the concavity of the trunk is directed to the left. A response of maximal strength is shown in G and H, illustrating a response to stimulation in the left side of the cerebellum. Photographs I and J show the type of reaction most often seen on stimulating near the sagittal plane and which consists of relaxation during stimulation followed by a rebound in which the two halves of the body respond in a similar manner, producing a symmetrical posture. The last pair of pictures, K and L, represents an ipsilateral forelimb response obtained from the medial part of the left cerebellar hemisphere.

involve fewer parts of the body but it is to be stressed that they are all of the same basic pattern and differ only in intensity.

While the majority of the reactions to cerebellar stimulation are composed of postures effected by the flexors and extensors of the limbs, it should be noted that pronation, supination, adduction and abduction of the limbs, torsion of the spinal column and rotation of the tail are not uncommon and that they appeared in the rebound as well as during the period of stimulation.

The level of decerebration, microscopically determined, was in no way related to the production of this type of response to cerebellar stimulation for the pattern, intensity and duration of the reactions were the same whether the brain stem was severed through the thalamus, at the rostral end of the midbrain or at the anterior margin of the pons. It seems certain that exclusion of the red nuclei does not alter cerebellar responses of this type for after transection of the brain stem caudal to the red nuclei responses of this kind were found to be just like those seen in decerebrate cats in which the red nuclei were intact.

Apart from the responses just described, there are others which can perhaps best be regarded as aberrant forms as, for example, those elicited when the stimulation was almost or exactly in the median sagittal plane. Often on such occasions the postures did not show the asymmetry of the typical reaction, neither in the first nor in the second phase. In other cases they were asymmetrical in one phase and symmetrical in the other. Such a reaction in which the right and left halves of the body responded in an identical fashion is shown in I and J.

When stimuli were applied in the lateral parts of the vermis or within the medial parts of the cerebellar hemispheres, postures of a strikingly different character were often produced. Throughout the duration of the stimulus the ipsilateral foreleg was either relaxed or slightly extended; on the rebound the limb was tonically flexed and sometimes adducted. This flexor rebound contrasted sharply with the strong extension of the limb obtained at the end of a stimulus placed a little more medially in the same side of the cerebellum. Other parts of the body took no part in either phase of this type of reaction which is illustrated in K and L. The tips of the stimulating electrodes in this case were in the left side of the cerebellum and it is only the left foreleg which is reactive. The frequent occurrence of these responses led to their recognition as the expression of a mechanism quite distinct from that activated by stimuli in the more medial parts of the cerebellum. The complete disappearance of this type of response as the electrodes approached the cerebellar peduncles makes it impossible to identify the efferent cerebellar tracts over which it is mediated but this ipsilateral forelimb reaction was not observed in any of the decerebrate cats in which the level of transection was caudal to the red nuclei. It was repeatedly obtained in one cat in which only the caudal poles of the

red nuclei were present but because of the possibility that these cells were not functional as the result of hemorrhage and trauma the evidence is not clear as to the participation of the red nuclei in this reaction. It must be taken into consideration that the path may be by way of the brachium conjunctivum and the fibers which branch from it below the level of the red nuclei and descend in the brain stem.

Some variations from the typical reactions to cerebellar stimulation which make their appearance after considerable damage has been done to the cerebellum by repeated punctures, have been described by Magoun, Hare and Ranson (in press) and need not be discussed here.

The cerebellar exploration which had been performed on decerebrate cats was extended to a series of cats with intact nervous systems. If one leaves out of account those decerebrate cats in which all of the cells of the red nuclei were removed and in which the isolated ipsilateral forelimb responses were missing, the reactions of the normal and decerebrate cats were so similar that only careful examination permitted the observer to differentiate between them. The minor differences which were consistently seen can be explained as the consequences of slight modifications of experimental conditions. The normal cats were anesthetized with nembutal which depressed the spontaneous and reflex activity of the animals throughout the experiment. Probably because of this, the strength of the responses was considerably decreased and full development of the postures of both phases was slower and more deliberate than in the decerebrate cats. Also the rebounds were of slightly shorter duration. Since all the cranial nerves were functional in the normal cats, cerebellar excitation caused ocular responses in addition to the reactions of the trunk and limbs which have been described. Usually there was a deviation of the eyes toward the side stimulated during the first phase and a rebound toward the opposite side at the end of the stimulus.

When the tips of the electrodes were in the fourth ventricle or in the medulla oblongata itself, stimulation produced twitchings of the muscles supplied by the cranial nerves. These responses were entirely different in character from those evoked by stimuli within the cerebellum.

Discussion. 1. Responses to cerebellar stimulation in lightly anesthetized cats with intact nervous systems have been found to be essentially similar to those obtained in a like manner in the monkey.

2. Identical reactions from lightly anesthetized cats with intact nervous systems and decerebrate cats show that the tonic background of decerebrate rigidity is not essential for the demonstration of cerebellar reactivity and support the suggestion made in regard to responses obtained from the monkey that these reactions to cerebellar stimulation are the same ones which have been inadequately described as the cerebellar inhibition of decerebrate rigidity.

3. Unimpaired activity of the medially situated cerebellar system after

transection of the brain stem behind the red nuclei demonstrates that a cerebello-rubral pathway is unnecessary for the mediation of its influence as was suggested by Cobb, Bailey and Holtz (1917) and indicates that its activity is discharged largely if not entirely by cerebello-bulbar connections, confirming Bernis and Spiegel (1925).

CONCLUSIONS

From the evidence derived from electrical stimulation of the cerebellum of the cat there seem to exist in that organ two neural mechanisms capable, when properly excited, of producing and maintaining biphasic postural responses of two very distinct patterns. The first of these is related to the entire axial and appendicular musculature; the influence of the second is restricted to the ipsilateral foreleg. Decerebration, even when the brain stem is severed caudal to the red nuclei, does not affect the activity of the first other than perhaps to add to its intensity, nor does decerebration appear to alter the expression of the second unless the transection entirely excludes the rubral apparatus, in which case the rebound flexion of the ipsilateral foreleg is no longer obtainable.

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THE SPINAL PATH FOR RESPONSES TO CEREBELLAR STIMULATION[1]

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A recent series of studies has indicated the important influence which an electrically excitable, medially situated cerebellar system is capable of exerting upon efferent centers for the contraction of axial and appendicular musculature. Investigation of the activity of this system in the decerebrate animal (Hare, Magoun and Ranson, 1936) has shown it to be unimpaired after removal of the red nucleus, which suggests that its influence may be mediated, in large part at least, by cerebello-bulbar and bulbospinal connections. In an effort to gain further information concerning the spinal pathways involved, the effect of lesions of the first cervical segment of the spinal cord upon the responses to cerebellar stimulation has been investigated in the cat.

METHOD. Using the Horsley-Clarke stereotaxic instrument (Ranson, 1934), responses to faradic stimulation of either side of the interior of the medial part of the cerebellum were observed following section of various parts of the spinal cord at the first cervical level in each of fifteen cats under light nembutal anesthesia. All of the experiments were performed acutely, the reactions being tested from fifteen to forty-five minutes following cord section. Control stimulation of the same foci in the cerebellum after exposure of the cord, but before section, had revealed characteristic responses. Suspension of the animals in the air by support of the head through the stereotaxic instrument and of the body by cords passed through the supraspinous ligament at the shoulder and pelvis, permitted complete freedom of movement of the limbs.

All spinal cord lesions were made on the left side. For hemisection and section of the lateral funiculus, the dorsal neck muscles were reflected and the caudal portion of the dorsal arch of the atlas was removed, permitting wide transverse section of the dura. For section of the ventral funiculus, a tracheal cannula was inserted, the larynx and esophagus were retracted to the right, the carotid sheath to the left and the vertebral muscles were reflected from the atlas and second vertebra. The ventral arch of the atlas and the odontoid process of the second vertebra were removed, and

[1] Aided by a grant from the Rockefeller Foundation.

the dura was sectioned in the midline from the body of the second vertebra to about the position of the rostral edge of the atlas. Retraction of the dura with fine forceps then gave an excellent exposure of the ventral aspect of the spinal cord. Cord sections were made with the aid of two fine scalpels, one straight with and the other at a right angle to the handle. The extent of each lesion was determined microscopically by a study of serial Weil-stained sections.

OBSERVATIONS. The response to stimulation of one side of the medial portion of the interior of the cerebellum is a biphasic one, one phase occurring during the period of stimulation, and the second phase being manifest as a rebound at the cessation of stimulation. This rebound consists, at its maximal strength, of a pronounced and longlasting extension of the ipsilateral limbs, a flexion of the contralateral limbs, and a contraction of the contralateral axial muscles. All of these postures are relaxed during stimulation and at the same time a contraction of the antagonists of these muscles often occurs. Variations in the responses consist chiefly in the extent to which inhibition or excitation is manifest, and in the extent to which all parts of the body participate. The effect of cord lesions on these responses may now be described.

Hemisection. Following hemisection of the first cervical segment of the spinal cord in each of four animals the reactivity of the limbs on the side of section was consistently abolished to stimulation of either the ipsi- or contralateral half of the cerebellum. The reactivity of the limbs opposite the side of section was consistently retained to stimulation of either half of the cerebellum. While possible "shock" effects associated with acute hemisection should not be overlooked, the results indicate that pathways which mediate the electrically excitable influences from either half of the cerebellum to the limbs on one side of the body are contained exclusively in the half of the spinal cord on that side.

With the establishment of a syndrome of hemisection, the attempt was made to delimit the significant portion of the hemisected area responsible for the deficit.

Section of the lateral funiculus. Following complete section of the lateral funiculus in each of two animals no impairment could be observed in the responses on either side of the body to activation of either half of the cerebellum.

Section of the ventral funiculus. Following section of the ventral funiculus which was complete to the midline in one animal and almost so in three others, the reactivity of the limbs opposite the side of section was not affected. The reactions of the forelimb on the side of section to stimulation of the ipsilateral half of the cerebellum were retained in all four of the animals, in two occasionally approaching the vigor of response seen before section, and in the other two being of consistently reduced intensity.

The reactions of the hindlimb on the side of section to stimulation of the ipsilateral half of the cerebellum were abolished in two animals, but were retained in the other two. The reactivity of the fore and hind limbs on the side of section to stimulation of the contralateral half of the cerebellum was considerably affected. Responses of the forelimb were consistently reduced in intensity, and lost all participation of flexor contraction in the rebound. Reactions were abolished in the hindlimb in two animals.

While section of the ventral funiculus has exhibited some variability of effect, it is apparent that this lesion causes an impairment not seen after lesions confined to the lateral funiculus of the spinal cord. It is obvious, however, that the effect of section of either the ventral or the lateral funiculus alone is not equivalent to that of hemisection of the cord.

Combined lesions of ventral and lateral funiculi. In three animals sections were made from the dorsal approach, interrupting not only the lateral funiculus but extending ventrally to involve about the lateral third of the ventral funiculus as well. The reactivity of the limbs opposite the side of section was not affected. In one of the animals the reactivity of the forelimb on the side of section was abolished to stimulation of either side of the cerebellum. In the other two animals with lesions almost identical to the first, the reactivity of the fore and hind limbs on the side of section was retained to stimulation of the ipsilateral side of the cerebellum, although usually reduced in intensity, and was abolished to contralateral stimulation.

In two animals sections were made from the ventral approach interrupting not only the ventral funiculus but extending dorsally to involve about the ventral half of the lateral funiculus as well. The reactivity of the limbs opposite the side of section was not affected. The forelimb on the side of section responded to stimulation of the ipsilateral half of the cerebellum only by a very slight rebound forward movement in the first animal and by a slow rebound extension and stepping in the second. To stimulation of the opposite half of the cerebellum this forelimb responded with a phasic rebound abduction in the first animal, and by a slight gain in extensor tone during stimulation in the second. In both animals, therefore, responses of the forelimb on the side of section were severely impaired. All reactivity of the hind limbs on the side of section was abolished.

Combined lesions of the lateral and ventral funiculi are seen, therefore, to produce a degree of impairment greater than that occasioned by sections confined to either funiculus alone, and lead to a deficiency in the reactivity of the limbs on the side of section which approaches that induced by hemisection of the cord.

The results clearly indicate that the pathways which transmit the effects of cerebellar stimulation to spinal levels are diffusely represented in both the lateral and ventral funiculi of the upper cervical cord, possibly with a

greater concentration in the ventral funiculus. It is of interest to note the similarity of this distribution with that of the reticulo-spinal tracts (Papez, 1926), more especially since the reticular formation of the brain stem has been emphasized as an important site of distribution of efferent fibers from the cerebellum (Allen, 1924, 1932; Rasmussen, 1933). Possible participation of descending connections from the vestibular nuclei should not be overlooked, however. The topographical data provided by the present experiments does not permit one to draw any final conclusions concerning the specific fiber tracts involved.

Interruption of ascending proprioceptive pathways does not appear to have been a factor of any importance in these experiments. Interruption of the dorsal spino-cerebellar tract in sections of the lateral funiculus has left the responses of the limbs on this side, as well as on the opposite side, unimpaired. Interruption of the ventral spino-cerebellar tract in many of the sections did not influence the reactions of the opposite limbs. It has been shown, moreover, in another investigation (Magoun, Hare and Ranson, 1936) that complete deafferentation of a limb does not affect its response to cerebellar stimulation other than to reduce the duration of rebound contraction.

SUMMARY

Following hemisection of the first cervical segment of the spinal cord, the responses of the limbs on the side of section to stimulation of either half of the cerebellum are abolished. The reactivity of the limbs opposite the side of section is retained.

Section of the lateral or ventral funiculus alone does not produce this deficiency, while combined but incomplete lesions of these funiculi cause impairment which approaches it.

It is concluded that at the upper cervical level, the pathways which transmit the effect of stimulation of either side of the cerebellum to spinal centers are diffusely represented in both the lateral and ventral funiculi of the half of the cord on the reactive side.

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BLOOD FLOW IN THE CIRCUMFLEX BRANCH OF THE LEFT CORONARY ARTERY OF THE INTACT DOG

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The thermostromuhr method of Rein has made possible an attack on the problem of coronary inflow since units can be placed on the main coronary arteries or their branches. Rein has done a series of experiments using his method to determine the blood flow in the right coronary artery of the dog. Hochrein and Keller, using Rein's method, studied the blood flow in the left coronary artery.

Previous studies on the coronary blood flow have been largely devoted to analyses of the factors responsible for variations in flow. Such investigations were of necessity confined to animals under general anesthesia. Every physiologist who has given the matter consideration realizes that data obtained from animals under general anesthesia represent at best only an approximation of what occurs in the normal, active, conscious organism. Consequently, in all our studies with the Rein stromuhr we have made every effort to determine the blood flow under as nearly normal conditions as we could devise. Our approach to the problem of coronary blood flow has been from a synthetic rather than from an analytical point of view. We are reporting here data on the blood flow in the circumflex branch of the left coronary artery of the intact dog in response to a variety of stimuli such as drugs, food, and exercise.

METHODS. In order to study the coronary blood flow it was necessary to develop a suitable unit for placing on the coronary arteries. It was likewise essential that the units be constructed so that they could be sterilized by boiling. A unit of the type used in our other experiments was not suitable for the coronary arteries. In order to have a minimal thickness of material interposed between the artery and the myocardium it was necessary to make the cuff that fits around the artery as thin as practicable. The multiplicity of lateral branches leading from the coronary arteries requires that the axis of the unit paralleling the vessel be as short as possible.

A unit was eventually designed to meet these specifications. It has been

our experience that units with internal diameters ranging from 1.0 to 2.5 mm. are of sufficiently variable size for use on the coronary arteries of dogs ranging from 6 to 20 kgm. in weight.

OPERATIVE PROCEDURES. Under ether anesthesia and with the usual aseptic technic, an adequate left lateral incision is made between the fifth and sixth ribs. The pericardium is incised over the region of the left coronary artery. The edges of the incised pericardium are then grasped and sufficient traction is exerted to bring the heart into the desired position. A serrefine is placed on the left auricular appendage, which is drawn out of the field of operation. The circumflex branch of the left coronary artery is then identified and dissected free. A short distance distally from the origin of the circumflex branch one or two lateral branches are usually given off, and the dissection is preferably made distal to these small branches. The artery is then placed in the unit and a ligature is passed under and around the middle of the unit and tied securely. The chest is then closed in the usual manner. Observations may be made if desired while the animal is still under anesthesia. We have usually made observations immediately after the operation and during the initial stages of recovery.

Effect of the unit on the general condition of the animal. As might be expected, the presence of a foreign body in the chest usually produces sufficient irritation to cause considerable pleural effusion until the leads and the unit are walled off. As a rule spontaneous drainage occurs and the animal progresses very well. It takes food and water, runs about the laboratory, and behaves in such a normal manner that a casual observer would not suspect the presence of the unit in the thorax. Some of the animals have gained weight during the course of the experiment. Typically the heart rate is considerably more rapid and irregular soon after the operation. In some cases a normal rate is resumed after a few days and the heart again becomes regular, whereas in other instances the increased rate and irregularity persist. The temperature taken by rectum may show a slight elevation above the normal range, which in the dog is from 100° to 102°F. (protocol 1).

RESULTS. *Blood flow immediately following operation.* In all of the animals studied the blood flow was greater immediately after the operation than it was following complete recovery from the ether anesthesia. The immediate postoperative values were frequently as much as three to four times those obtained twenty-four hours later. Because of the necessary trauma incident to placing the unit on the vessel, it cannot be stated that the anesthetic was solely responsible for the greater blood flow. Representative data are given in protocol 1.

Blood flow in the quietly resting dog. Following operation, daily (except Sunday) observations were usually made while the animals rested quietly on a table for an hour or more. In a number of instances we obtained daily

PROTOCOL 1

Coronary blood flow in the quietly resting animal

DOG	WEIGHT	TIME AFTER OPERATION	PULSE RATE, BEATS PER MINUTE	AVERAGE BLOOD FLOW	RECTAL TEMPERATURE	CONDITION
	kgm.	*hours*		*cc. per minute*	*°F.*	
5	14.5*	4		163		
		6		114		
		24	86 to 108	89	102.8	Good
		48	96 to 112	84	102.3	Eats well
		72	110 to 114	82		Eats well
		96	102 to 112	75		Eats well
		144	86 to 100	80	102.6	Eats well
		192	104 to 108	38	103.1	Eats well
		216	70 to 80	26	103.4	Eats well
		240	70 to 82	43	103.0	Eats well
		336	64 to 78	34	102.0	Eats well
10	15.4	Immediately		115		
		2		85		
		4	112	65	100.2	Good
		24	160 to 200	82	101.8	Eats well
		48	200+	63	102.0	Eats well
		120	150 to 160	76	102.1	Eats well
		144	102 to 120	91	101.6	Eats well
		168	98 to 106	80	102.0	Eats well
		192	74 to 88	85	102.0	Eats well
		216	76 to 86	80	101.0	Eats well
12	17	Immediately		90		
		24	110 to 130	66	103.5	
		48	130 to 140	75	103.3	
11	18.2	Immediately		74		Excellent
		4		61		
	18.2	24	180 to 186	50	104.2	
		48	160 to 200	53	103.7	
	19.0	72	120 to 140	64	102.6	
	20.0	96	102 to 116	76	102.2	
8	9.6	Immediately		77		
		6		28		
		24	118	23	103.5	
		48		21	103.5	Developed distemper
		72	114 to 120	30	104	
		144	96 to 102	40	103.8	
		192	142 to 200	47	105.2	Died

* This animal was killed 5.5 months after placing unit on coronary artery and necropsy was performed immediately: The unit was free from the coronary artery, which had been obliterated. The proximal end of the vessel had become occluded and was imbedded in a small mass of scar tissue. The unit was found on the edge of the pericardium imbedded in a small nodule of adhesions. There was no infection within the pleural cavity. Grossly the heart appeared normal but, on palpation, a firm area was found at the site where the unit had been applied.

values that fell within the experimental error of the method (10 per cent). In one series of observations we obtained comparable rates of flow on each succeeding day up to ten days after the unit was placed on the coronary vessel. Occasionally there occurred a reduction in the rate of blood flow by the fifth or sixth postoperative day. We have taken a gradual decrease in coronary flow to indicate that the lumen of the vessel is reduced, probably as a result of tissue reaction to the unit (protocol 1).

Complete occlusion of the coronary artery occurred in two cases within a week after operation. One of the animals was running about the laboratory when the occlusion occurred; it appeared to have been in splendid condition and had manifested intense interest in its surroundings, but suddenly it leaped, barked and succumbed. Necropsy immediately after death revealed complete occlusion of the coronary vessel to which the unit was applied.

Effect of certain drugs on coronary blood flow. Epinephrine. It has been demonstrated by numerous investigators that injections of epinephrine cause an increase in blood flow through the coronary vessels of anesthetized animals. Consequently we were interested in determining the effect of epinephrine on the coronary inflow of the intact dog. Repeated injections of 0.05 to 0.1 cc. of a 1 to 1000 solution of epinephrine caused a marked transient augmentation of from two to four times the control values. The augmentation in flow did not last longer than seven minutes in any of our experiments. As a rule the major effect on the coronary flow had terminated three minutes after injection of the drug, thus paralleling the known effect of such doses of epinephrine on the blood pressure of the dog.

Nitrites. 1. Nitroglycerine: Repeated observations under the conditions of our experiments have indicated that nitroglycerine in intravenous doses of 0.22 mgm. results in a temporary increase in coronary blood flow which at its maximum is about twice the control value. However, the increase is of short duration, since it usually is over in four or five minutes after the injection.

2. Amyl nitrite: The results of our initial experiments with this drug were open to the criticism that the nasal passages of the animals were not cocainized before inhalation of the amyl nitrite vapor was begun. It is conceivable that reflexes resulting from the stimulation of the respiratory passages might affect the coronary flow, such as movements or failure of the animal to respire normally. In a series of later observations the nasal passages of the dogs were cocainized by means of cotton swabs soaked in a 5 per cent solution of cocaine. Additional cocaine solution was introduced by syringe. After fifteen minutes the swabs were removed and the mask was placed over the dog's muzzle. The mask did not cause an appreciable change in coronary flow. After the mask had been in place for a short time an ampule of amyl nitrite was broken in it and inhalation followed

without alteration in the depth of respiration. Within two minutes after the amyl nitrite was introduced the coronary blood flow had increased to about twice the control values. Although the animal continued to breathe the drug, the blood flow did not remain at twice the control level for more than two minutes when it declined to a value 20 to 30 per cent above the control. On removal of the mask the blood flow approximated the control values within five minutes. In one experiment a second ampule was broken in the mask six minutes after the effect of the first ampule had been dissipated. Only a slight elevation in coronary blood flow followed. From a control of 70 cc. the blood flow increased to only 86 cc. per minute.

PROTOCOL 2

Effect of inhalation of amyl nitrite on coronary blood flow

(Dog number 12, weight 17 kgm.)

TIME	BLOOD FLOW		REMARKS
	Control	During inhalation	
	cc. per minute	*cc. per minute*	
11:25	60		
11:40	63		Nasal passages cocainized for 15 minutes
12:10	66		
12:15	71		Mask on
12:18	71		Ampule of amyl nitrite broken in mask
12:18.5		103	
12:19.5		137	
12:21.5		88	
12:23			Mask off
12:25		68	
12:31		70	Mask on; second ampule amyl nitrite broken in mask
12:32		86	
12:33		80	
12:34		86	
12:36		70	Mask off

These experiments clearly indicate that amyl nitrite is capable of producing a temporary increase in coronary blood flow in the intact dog, but it appears that the dog is refractory to a second dose when it is given too soon after a previous one (protocol 2).

Thyroxin. In a previous paper (3) we have shown that the blood flow in the femoral artery of a series of six dogs was increased from 205 to 327 per cent over the control figures.

Because of the time required to produce hyperthyroidism, an adequate study of the effect of thyroxin on coronary blood flow has not been possible by the use of previous methods. In a series of experiments we have

studied the changes in coronary blood flow produced by intravenous injections of thyroxin. Following operation, observations were made on successive days to establish control values. When this had been done, an injection of 1 mgm. of thyroxin for each kilogram of body weight was given intravenously. In one experiment a second injection was given on the following day. There was no significant change twenty-four hours after the injection, but within forty-eight hours the blood flow had markedly increased, having in two experiments reached the maximum, which was 230 and 244 per cent, respectively, above normal. The accidental death of one of these animals terminated the experiment. The coronary blood flow of the other had fallen from 244 per cent above the control flow at forty-eight hours to 61 per cent ninety-six hours after the injection of thyroxin. In a third animal the maximal flow, 142 per cent above normal,

TABLE 1

Effect of thyroxin on coronary blood flow

DOG	WEIGHT	CONTROL BLOOD FLOW	HOURS AFTER GIVING THYROXIN	INCREASE IN BLOOD FLOW
	kgm.	*cc. per minute*		*per cent*
11	18.2	70 to 78	48	230
12	17	112 to 127	24	0
			48	56
			72	100
			96	142
			120	36
13	15	80 to 83	24	17
			48	244
			96	61

was not reached until ninety-six hours after the injection of thyroxin, and in 120 hours the flow was about 36 per cent above the control values.

It is therefore evident that thyroxin causes a marked increase in the blood flow through the coronary arteries which is comparable to the augmentation observed in the femoral artery in response to this drug (table 1).

Effect of digestion on coronary blood flow. In a previous publication (4) we have reported that during digestion the blood flow in the somatic as well as the visceral vessels is increased from 35 to 127 per cent in response to the digestion of a mixed meal. Consequently it was to be expected that the additional work thrown on the heart during digestion would be reflected in an increased flow through the coronary arteries, and such is the case. We have observed an increase of as much as 84 per cent in the coronary blood flow of dogs during the digestion of food.

Effect of exercise on coronary blood flow. The influence of exercise on the coronary blood flow has been considered by physiologists for many years. Many calculations have been made as to the requirements of the heart during strenuous exertion. A series of experiments has been performed in order to obtain information on this question.

For investigations of this nature dogs are trained to walk on a treadmill, the belt of which is driven by a motor. When the belt is moving in a horizontal position it is necessary for the animal to walk rapidly and moderate exercise is thus required. The belt can be set at any desired angle from the horizontal to about 30°. When set at an angle of 15°, a dog weighing 15 kgm. performs work amounting to approximately 20 foot pounds per

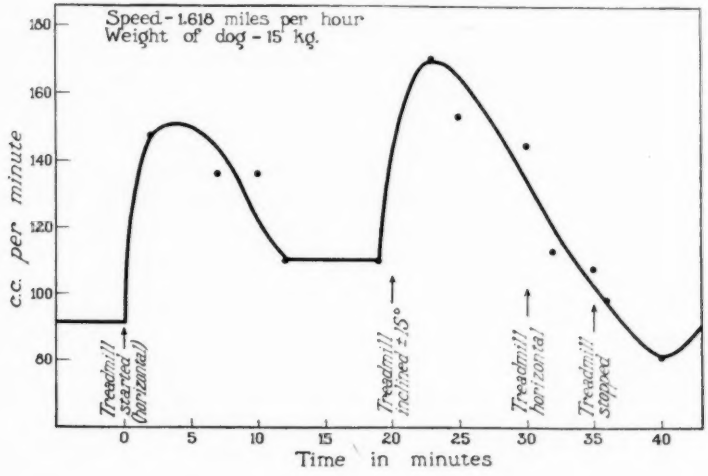

Fig. 1. Bloodflow in circumflex branch of left coronary artery before and during exercise on treadmill.

second. The speed of the treadmill can be varied from 1.152 to 3.756 miles per hour. We have routinely run the machine at a speed of 1.618 miles per hour.

In each experiment the coronary blood flow was observed first while the dog was resting quietly on the table, then while standing on the treadmill, and finally while the mill was running in a horizontal position or at an angle of 15°. There was not a great difference between the values for blood flow whether the animal was standing quietly or was lying quietly. However, on starting the treadmill there was an immediate rise in coronary flow which attained maximal values in about five minutes after which it gradually subsided to a much lower level in the succeeding five minutes.

The flow remained relatively steady at the lower level until the amount of exercise was increased. This was easily accomplished by rapidly elevating the treadmill to the desired angle, whereupon the flow again rapidly increased for about five minutes, when it declined to a lower level but remained above the second level reached during the previous increment of exercise. If the treadmill was then lowered to the horizontal, the flow rapidly diminished until it reached the second level about which it fluctuated. When the mill was stopped, the flow rapidly returned to the control level or even fell below the control values before stabilizing. Although every experiment did not reproduce the foregoing description in all details, nevertheless each experiment was comparable in the essential features to the one just described (fig. 1).

Coronary blood flow and weight of the heart. Various figures are given for the amount of coronary blood flow for each 100 grams of heart weight, but in general it should be understood that such data are of questionable value unless the conditions under which they were obtained and the physical state of the animal are indicated.

As has been stated, all the observations reported in this paper were made on the flow in the circumflex branch of the left coronary artery. According to Anrep, Blalock and Hammouda, the flow in this vessel represents about 50 per cent of the total inflow by way of the coronary arteries. When the total coronary flow is taken as twice the flow in the left circumflex artery and a calculation is made in terms of 100 grams of heart weight, there is no consistent relation seen in our data since, expressed in this manner, the values vary from 76 to 176 cc. All of the values for blood flow used in the calculations were obtained while the animals were lying quietly on the table.

Heart rate and coronary flow. Investigators have been divided on the question of the relation of heart rate to coronary flow. In our experiments we have not obtained any convincing evidence that there is a consistent relationship between pulse rate and coronary flow. The pulse rate may vary within wide limits while the coronary flow remains relatively constant (dog 10, protocol 1).

SUMMARY AND CONCLUSIONS

By the use of the thermostromuhr of Rein, experiments have been made on the blood flow in the circumflex branch of the left coronary artery of the intact dog. Observations have been made over periods as long as fourteen days.

Epinephrine causes a transient but marked increase in coronary blood flow amounting in some experiments to as much as four or five times the control values. The coronary blood flow is doubled by administration of nitroglycerine or amyl nitrite, but the effect is of short duration. In response to appropriate doses of thyroxin, increases in coronary flow as

great as 244 per cent above the control values were observed forty-eight to ninety-six hours after injection.

During the digestion of a meat meal the coronary flow was increased to a degree comparable to what has been observed in other vessels of the body. Exercise on a treadmill produced an initial rapid augmentation in coronary flow, which declined to a lower level as the exercise continued but additional work induced by changing the angle of the treadmill again caused a temporary increase in coronary flow, which declined to a lower value as the same degree of exercise continued.

A significant correlation between heart weight and coronary flow or between pulse rate and coronary flow was not found.

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CAROTENE AND ASSOCIATED PIGMENTS IN MEDULLATED NERVE

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It has long been known that the yellow pigmentation of the normal vertebrate nervous system is due primarily to the lipochromes (Meschede, 1872; Mayer, 1876; Rosin, 1896; Neumann, 1909; Dolley and Guthrie, 1918). With the discovery that beta carotene is provitamin A this pigment assumed new importance in metabolic processes, and in view of its wide distribution in nervous tissue it is desirable that its rôle in nerve processes be studied. Its possible rôle as an oxygen transferring substance was suggested by Arnaud (1889) and Willstätter and Miegs (1907). That this suggestion may be of importance in connection with oxidative processes in nerve has been pointed out by Monaghan and Schmitt (1931). Schreiber (1932) discussed the rôle of chromolipid pigments in the ganglia of gasteropods. It has also been shown recently by Wald (1935) that carotene and vitamin A, in combination with certain proteins, together with the pigment retinene, are essential in chemical processes taking place in the rods of the retina.

Since nerve processes are most easily studied in cold-blooded peripheral nerve we have chosen the sciatic and brachial nerves of bullfrogs as material. In the present paper data are presented on the normal level of carotene and related pigments in these nerves, together with the effect of prolonged fasting and of feeding experiments.

METHOD. The combined sciatic and brachial nerves weighing about 1.5 gram obtained from three large bullfrogs sufficed for a single determination. The nerves were dissected free of extraneous tissue, weighed, minced, plunged into 20 cc. of 95 per cent ethyl alcohol, and allowed to stand in the refrigerator for 48 hours. The liquid was then decanted and to the residue were added 20 cc. saturated alcoholic potassium hydroxide and boiled for about five minutes. This results in complete solution of the tissue. After cooling, this liquid was combined with the alcoholic fraction and transferred to a separatory funnel. To this were added 200 cc. distilled water and 75 cc. ether and the mixture shaken. The carotenes, cryptoxanthin, xanthophyll and vitamin A collect in the ether layer. The water layer was drawn off and extracted with ether until no further color was removed.

The aqueous material was kept for vitamin G determinations. The combined ether fractions were repeatedly washed with water until the wash water was neutral to litmus. The ether fraction was then dehydrated with sodium sulphate and evaporated to dryness in a vacuum desiccator.

The various carotenoid pigments were separated as follows. The residue was dissolved in 25 cc. petroleum ether and washed repeatedly with 92 per cent methanol. The methanol fraction containing the xanthophyll was separated off. The petroleum ether fraction was then treated with

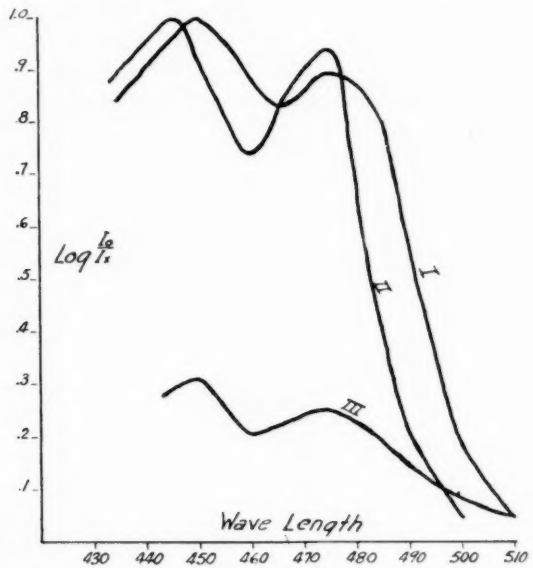

Fig. 1. Absorption curves of carotene in petroleum ether.
I—Beta carotene, 1.40 mgm. per liter; depth, 3 mm.
II—Alpha carotene, 1.40 mgm. per liter; depth, 3 mm.
III—Carotene in nerves; depth, 30 mm.

calcium carbonate, thus separating the carotene from the cryptoxanthin, the latter being adsorbed on the calcium carbonate. This fraction after elution was determined qualitatively by its absorption spectrum.

The carotene and xanthophyll were determined colorimetrically against standards freshly made from the pure compounds.[1] The carotene determinations were also checked spectrophotometrically.[2] A typical curve for

[1] Pure α and β carotene were obtained from the SMA Corporation. Xanthophyll was obtained from American Chlorophyll Company.

[2] We are greatly indebted to the Ralston Purina Company for the use of their Bausch and Lomb spectrophotometer.

nerve carotene is shown in figure 1, along with curves of pure alpha and beta carotene for comparison. Nerve carotene appears to contain a mixture of the two forms. The maxima of the nerve preparation were not as distinct as those from pure carotene but lay essentially in the same general region. The values obtained from the extinction coefficients agreed with those from the colorimetric method within about 0.5 gamma per gram. Vitamin A was determined colorimetrically by a modified Carr Price method.

RESULTS. For physiological experiments on frog nerves the animals are commonly kept in the ranarium often for a period of weeks or months

TABLE 1

Carotene and associated pigments in nerves of fasting bullfrogs

NUMBER OF BULLFROGS EXAMINED	TEMPERATURE OF RANARIUM	PERIOD OF FASTING	WEIGHT OF NERVE	CAROTENE	VITAMIN A	OTHER PIGMENTS FOUND
	°C.	*days*	*grams*	*γ per gram*	*units per gram*	
8 (controls)	15	0	3.01	13.9	21	21γ per gram Xanthophyll Cryptoxanthin
3	15	22	1.23	6.9	8	Xanthophyll
3	15	36	1.40	1.3	<2	Xanthophyll
12 (controls)	15	0	3.90	8.3	25	Xanthophyll
4	15	16	1.53	1.8	<2	Xanthophyll
4	15	22	1.42	1.6	<2	None
2	15	43	0.85	0.5	None	None
12	25	0	2.7	11.6	Present	Xanthophyll
4	25	0	1.8	9.6	Present	Xanthophyll
3	25	30	1.3	0.5	None	None
3	25	40	1.66	0.5	None	None

without feeding. Aside from determining the level of these pigments in the nerves of normal frogs it was, therefore, also desirable to obtain information on the changes in these pigments and in vitamin A which might result from such captivity. Each fresh shipment of Louisiana bullfrogs was kept in a separate ranarium. Eight to twelve frogs were killed immediately upon arrival and of the remainder, groups of three or four frogs were killed at various intervals of time later. In two series of experiments the fasting frogs were kept under "winter" conditions, the temperature of the ranarium being about 15°C. In one series the frogs were artificially "summered," the temperature of the ranarium being about 25°C.

The results are summarized in table 1. Obviously even under "winter"

conditions the carotenoid pigments and vitamin A are greatly depleted in three or four weeks of captivity without feeding. This depletion appears to be accentuated when the frogs are kept under "summer" conditions. This was also indicated by changes in skin color. The data in table 1 do not reveal the extremes between which the pigmentation varied because of the necessity of using the combined nerves of a number of frogs for each determination. The nerves of one frog may have been quite yellow while those of the next may have been practically white. An upper limit for the carotene level of the nerves of freshly caught frogs appears to be about 30 gammas per gram.

It has long been known that while the nerves of frogs kept for long periods in the ranarium without feeding are capable of conducting the impulse in fairly normal manner, abnormalities may be demonstrated.

TABLE 2

The effect of carotene and xanthophyll feeding on nerve pigmentation

NUMBER OF BULLFROGS EXAMINED	FASTING PERIOD PRIOR TO FEEDING TEST	FEEDING PERIOD	PIGMENT FED DAILY	WEIGHT OF NERVES	CAROTENE	VITAMIN A
	days	*days*	*mgm.*	*grams*	*γ per gram*	*units per gram*
3	43	0	0	1.3	None	None
4	45	7*	0.5 carotene	1.80	5.7	22
2	50	7	1.0 carotene	0.92	9.2	Present
2	45	12	0.5 carotene + 0.5 xanthophyll	0.83	0.5**	40

* Tests made 8 days after last feeding.

** 0.5 γ per gram of xanthophyll found.

Thus Froehlich (1903) found that unless such kept frogs are fed, complete recovery of the action potential following readmission of oxygen after an asphyxial period is impossible. The present experiments demonstrate the serious depletion of carotene and vitamin A in the nerves of animals kept for extended periods without food. It seems obvious that nerve physiologists should give more attention to the diet of the frogs used in their experiments, particularly in the case of bullfrogs which, because of their hardiness, are frequently kept for months in the ranarium before being used in experiments.

Particularly with a view to further experiments on the physiological rôle of these pigments in which it would be desirable not only to deplete the nerves of pigment but also to increase the pigment to or above the normal level, frogs were fed quantities of carotene and of xanthophyll for a period of a week or so and the storage in the nerves determined. It will be seen

from table 2 that considerable storage of carotene occurred in animals which had been completely depleted by a preliminary period of fasting. In some of these animals the nerves were deep orange in color. It is significant that of the group fed a mixture of carotene and xanthophyll the pigment was apparently converted largely to vitamin A rather than being stored as such. The rôle of xanthophyll in this interconversion is not clear.

SUMMARY

1. The carotene and vitamin A content of the peripheral nerves of bullfrogs has been determined.

2. The level of these pigments in nerves of frogs kept in the ranarium without food drops considerably and may be entirely depleted in three to four weeks depending on the temperature.

3. Daily feeding of carotene causes considerable storage in the nerves within a week. If xanthophyll is also fed there is considerable conversion to vitamin A in the nerves.

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GERMINAL RESPONSE (IN MALE MICE) TO ENVIRONMENTAL CONDITIONS[1]

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Statistical data have been presented by Mills which show that human fertility in any given population is highest between 40°F. and 65°F. and that it declines sharply at mean temperatures above 70°F. or below 40°F. (1), (2). He has also shown that sexual maturity has an earlier onset in the human female in the more invigorating zones than in those less stimulating (3). Results comparable in all essentials to the human data have been obtained in this laboratory with albino mice (4). Female descendants of mothers kept under stimulating environmental conditions bore young sixteen days earlier than did their mothers. Coincidentally, it was found that the fertility of male mice, and also the position of the testes, were influenced by the climatic environment. Conditions of steady moist heat permitted only a very low degree of fecundity, although the testes rested in large loose scrotal sacs. On the other hand, retention of the testes intra-abdominally did not produce sterility under invigorating environmental conditions. These findings, obtained by matings, have been augmented by histological studies which reveal a morphological basis for this very marked dissimilarity of sexual functionings in animals exposed to different environmental conditions.

EXPERIMENTAL CONDITIONS AND RESULTS. The data which follow were obtained by examination of the testes of mice from the experimental groups previously described from this laboratory (4).[2] Mating results

[1] This work, carried on during 1933 and 1934, was aided financially by the National Research Council and the Ella Sachs Plotz Foundation. I wish to take this opportunity to express my appreciation to these donors for their aid.

Data herein presented were taken from a Ph.D. thesis, University of Cincinnati, 1934.

[2] The experimental groups consisted of control room group, a cold room group, a hot room group and two groups of animals that were shifted daily between the hot and cold rooms. For convenience, these shifted groups are considered together here because their reactions to great temperature variability were alike. The control room was kept at 70–80°F. The cold room had a temperature range of 60–68°F. and in the hot room the temperature ranged from 88–92°F. with the humidity near 75 per cent. The construction of these constant temperature rooms has been described elsewhere (5).

TABLE 1

1. At 8 weeks of age	
Cold room males (13)[3]	Spermatozoa present, many very active tubules, pyknosis, peripheral tubules vacuolated, no giant cells
Hot room males (12):	Spermatozoa present, lumina large, peripheral vacuolization, pyknosis, giant cells rarely observed.
2. At 12 weeks of age	
Cold room males (13):	Same as at 8 weeks
Hot room males (15):	Spermatozoa present, varying stages of activity, peripheral tubules vacuolated, giant cells more frequent
3. At 16 weeks of age	
Cold room males (9):	Spermatozoa present, fairly active but thin epithelium, pyknosis, giant cells rarely observed
Hot room males (3):	Spermatozoa present, thin epithelial linings, increase in giant cells, extensive vacuolization of peripheral tubules
Change group males (8):	Spermatozoa present, varying stages of activity, peripheral tubules vacuolated, giant cells occasionally observed, small tubules
4. At 20 weeks of age	
Cold room males (16):	Spermatozoa present, epithelial linings active but thin, peripheral tubules vacuolated, pyknosis
Hot room males (9):	Few, if any, spermatozoa; more advanced degeneration, giant cells numerous
Change group males (10):	Spermatozoa present, thin epithelial linings, varying stages of proliferation, peripheral tubules vacuolated, few giant cells, small tubules
5. At 25 weeks of age	
Control room males (6):	Tubules normal and highly active.
Hot room males (5):	Some moderately active tubules with spermatozoa present, interstitial tissue very prominent, peripheral tubules vacuolated
Change group males (10):	Few spermatoza, peripheral tubules vacuolated, giant cells frequent, small tubules
6. At 31 weeks of age	
Change group males (9):	Tubules very small, extensive degeneration, giant cells frequent, tubular shrinkage
7. At 38–52 weeks of age	
Hot room males (5):	Same as at 25 weeks
Cold room males (3):	Same as at 20 weeks
Control room males (8):	Same as at 25 weeks
Change group males (6):	Same as at 31 weeks

showed that the cold room males were equally as fertile as control room males although the testes of the former resided within the abdominal cavity so long as the animals were kept in the cold room, while on the contrary the sex glands of the hot room and change group males rested in large loose scrotal sacs and these animals had a very low fertility.

Comparable numbers of animals from each group were sacrificed at similar ages. The animals were killed quickly either by a blow on the head or by ether. The testes were studied histologically after fixation in 4 per cent formaldehyde and staining with hematoxylin and eosin.

A brief summary of the microscopic findings in the four groups is presented in table 1.

Nuclear material was pyknotic in all tubules except those of the control room mice kept constantly in the control room. Also the cytoplasm of the control room animals stained more deeply with eosin, under the same technique, than did that from any other group of mice. The hot room and cold room males presented similar testicular tubules up to the age of twelve weeks; most of the peripheral tubules were vacuolated, nuclear material was pyknotic and varying degrees of proliferation were observed (figs. 1 and 2). From this age on the hot room males showed more extensive vacuolization, the tubules had thin epithelial linings and contained a large number of giant cells. Such degeneration is illustrated by figure 1, which portrays a cross-section of the testes from a hot room male at 20 weeks of age. Between the ages of eight to twelve months, giant cells were not so abundant, yet many tubules were bare, the interstitial tissue very prominent and the degree of activity quite variable. Spermatozoa were present in some tubules but not usually so where there was extensive vacuolization.

The germinating epithelium of the cold room group males was usually thin, but many spermatozoa were present. Giant cells were rarely observed. Nine cold room males (five months of age) were placed in the hot room, kept there for varying periods and then killed. Within three days after transfer to the hot room, the testes of these mice had descended into deep scrotal sacs. Those animals, originally from the cold room, which were killed after being in the hot room for one month, presented a highly degenerated germinal tissue with extensive vacuolization, many giant cells and few, if any, spermatozoa (fig. 4). Following a sojourn of two months in the hot room there were fewer giant cells and an increase in all types of germinal cells save spermatozoa. Hence, animals transferred from an invigorating environment to one of humid heat suffer an acute loss in germinating epithelium soon after their change in climate. In due course these transferred animals become adapted to their new environment and the tubular linings are partially regenerated, but mature germinal cells are not produced. This regeneration is complete and more rapid when the

Fig. 1 Fig. 2 Fig. 5 Fig. 6

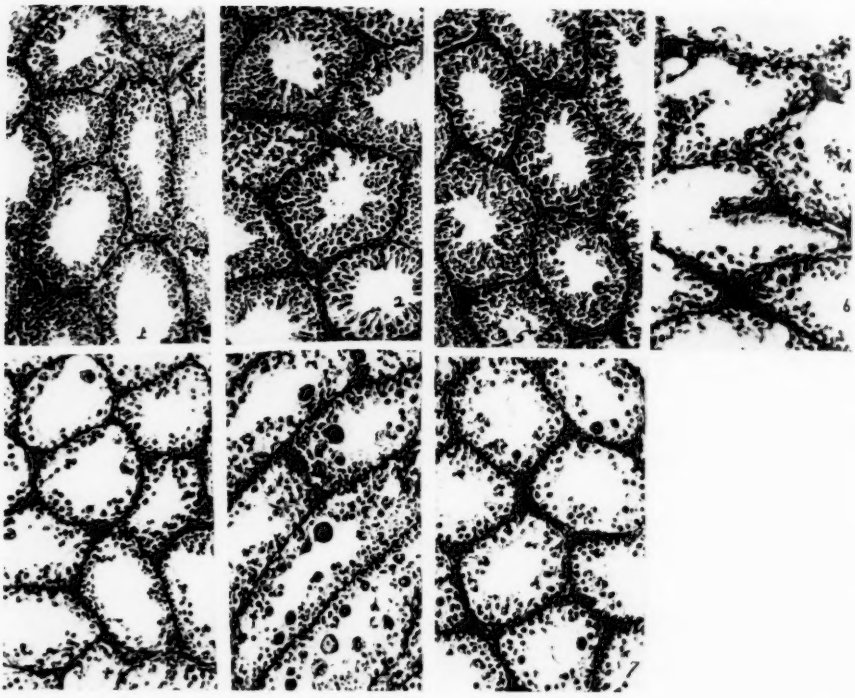

Fig. 3 Fig. 4 Fig. 7

Fig. 1. Hot room male no. 501. Age 9 weeks. Body weight 16.0 grams. (200×.)

Fig. 2. Cold room male no. 621. Age 8 weeks. Body weight 23.3 grams. (200×.)

Fig. 3. Hot room male no. 43. Age 20 weeks. Body weight 17.0 grams. (200×.)

Fig. 4. Cold room male no. 117. Killed after 36 days in the hot room, at which time the animal was 6 months old. Body weight was 26.3 grams when transferred to the hot room, and 23.3 when killed. Much hair was lost from the abdomen. (200×.)

Fig. 5. Cold room male no. 101. This animal was placed in the hot room for 26 days, and returned to the cold room for one week before being killed. Age at death 6 months. (200×.)

Fig. 6. Control room male no. 238–9. Age nine months. Placed in hot room for 10 days and killed upon removal therefrom. Body weight when removed from control room was 28.9 grams; 27.9 grams at time of death. (200×.)

Fig. 7. Change I group. Male no. 229. Weight 25.2 grams. Age at death 6 months. (200×.)

animals are returned to their original habitat. One cold room male was placed in the hot room for twenty-six days and at the end of this period was returned to the cold room for one week before being killed. In spite of the fact that the testes promptly returned to their abdominal position when the animal was replaced in the cold room, regeneration occurred soon. The germinal epithelium was fairly thick, although it was almost devoid of spermatozoa and contained a few giant cells (fig. 5).

The control room males, when placed in the hot room, reacted in the same manner as did those of the cold room so treated. Ten days in the hot room was the shortest exposure of the control room or cold room males to the humid heat. At the end of this time, the germinal tissue has undergone extensive degeneration, and contained a great number of giant cells. No spermatozoa were present (fig. 6).

The testes of the males shifted daily between the hot and cold rooms showed various degree of activity, with occasional giant cells, up until the age of approximately six months. At this time most of the tubules showed much degeneration with few spermatozoa, many giant cells, and prominent interstitial tissue (fig. 7). The tubules were quite small in all of the animals of this group. We see here in the degenerated germinal tissue the reason for so very few pregnancies resulting from the mating together of males and females that were shifted daily between the hot and cold rooms. These males, and those of the hot room, possess scant amounts of actively proliferating germinal epithelium, while a most intense loss of this tissue occurs quickly after the transfer of control or cold room mice to the hot room.

Discussion. These findings provide strong presumptive evidence that the physical environment markedly influences the histology, as well as the activity, of the germinal epithelium of the testes. In every instance, exposure to humid heat led to the degeneration of this tissue, accompanied by the production of many giant cells. Large, loose scrotal sacs, such as those possessed by the hot room and shifted males, are considered by Sundstroem as an adaptation to facilitate heat loss by radiation (6). Moore and co-workers (7) and Fukui (8) found that when the testes were kept at the temperature of the peritoneal cavity, the germinating epithelium was rapidly destroyed. In Moore's experiments, restoration of the germinal epithelium occurred only when the testicular temperature was lowered a few degrees below that of the body. In all mammals examined by him and Quick (9), the scrotal temperature was below that of the peritoneal cavity. In the experiment here recorded the cold room mice retained their testes intra-abdominally and were fully as fertile as control ones. Transfer of cold room or control room mice to the hot room led to an early epithelial degeneration, even though the testes of the former group of animals descended into scrotal sacs while they were in the hot room.

However, a prolonged stay in the hot room led to the regeneration of all types of germinal cells except spermatozoa. Such regeneration was accomplished even more quickly by the return of these transferred animals to their original habitat, where mature germinal cells also were produced.

The experimental conditions under which these animals were kept affected primarily the ease of body heat loss. Body heat production is definitely suppressed under conditions of humid warmth, and this suppression is probably based on a decline in function of the adrenal cortex. It is not advisable to enter here into a review of the literature showing a close relationship to exist between adrenal and sex gland functionings. In general it has been well shown that the two sets of glands are intimately linked. Hence it is in no way surprising that we should find suppression of fertility and gonadal activity by environmental conditions that render difficult the loss of body heat.

In the human being, sudden exposure to humid heat frequently leads to functional suppression of the adrenal gland (10), while it has also been shown with experimental animals that the metabolic rate and heat production decrease as the temperature of the environment is raised (11). Hence, under the conditions of this work, it seems probable that the state of activity of the germinating epithelium is more intimately dependent upon the animal's metabolic level than upon the anatomical position of the testes. This point is clearly illustrated in the immediate desquamation of the spermatic tubules of animals suddenly exposed to humid heat, since the desquamation takes place in spite of a migration of the testes from their intra-abdominal position down into deep scrotal sacs.

The suppression in the metabolism of the germinal tissue affects both the nuclear and cytoplasmic materials. Mature spermatozoa, the most labile products of this epithelium, rapidly disappear, but the nuclear material of the younger germinal cells continues to divide long after the cytoplasm ceases to do so, and thereby results in the production of multinuclear masses of protoplasm. This germinal suppression seems most probably associated with a general metabolic subsidence as the external humid heat makes body heat loss difficult. The close linkage of adrenal cortical activity to heat production in the body and also to activity of the sex glands, intensifies the responsiveness of these glands to external temperature conditions.

SUMMARY

1. The germinating epithelium of male mice kept under conditions of constant humid heat undergoes degeneration, accompanied by giant cell formation. Few actively proliferating tubules are present between the ages of four and twelve months, although the testes reside in deeply pendent scrotal sacs.

2. Mice adapted to an invigorating environment retain their testes

intra-abdominally and have active germinal tissue. Submission of such males to humid heat leads to an abrupt degeneration of the testes. Control mice show a similar reaction to humid heat.

3. After exposure to humid heat, the germinal tissue of cold room or control room males is regenerated more rapidly when these animals are returned to their original experimental climate than when kept for prolonged periods under the heat conditions.

4. Microscopic studies of the testes verify the fertility findings previously reported, viz., that the sexual functions are quickly and markedly affected by changes in the stimulating character of the environment. Moist heat, by rendering body heat loss difficult, produces a prompt suppression of fertility and of microscopic evidences of germinal activity.

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COMPONENTS OF THE ELECTRICAL RESPONSE OF THE OPTIC CORTEX OF THE RABBIT[1]

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This paper deals with the form of the electrical responses of the optic cortex to stimulation of the optic nerve, as recorded from different depths in the cortex. Histological material from the animals upon which the experiments were performed is being further studied, with a view to correlating the electrical responses obtained with the structure at the specific levels led from. It is to be presumed *a priori* that the spatial sequence of elements successively activated in the cortex will not be simply linear, and that the temporal sequence of their responses will not be a simple succession. In spite of this, a first approach seems feasible through considering the cortex as a modified peripheral nerve trunk, in which certain elements may terminate, and others originate, between any two electrodes placed along the pathway of the impulse. This "nerve" will have different effective cross-sections at different levels, and complicated shunts of extraneous tissue.

To make the conditions as simple as possible, the rabbit was chosen as experimental material because its cortex is of the lissencephalic type, that is, without fissures. The optic nerve was stimulated directly, after removal of the eye, by single electric shocks or 2 ms. galvanic currents, each stimulus resulting in a single volley of impulses in parallel fibers passing over the pathway as nearly synchronously as possible. Light ether anesthesia was employed. The pathway then consists of optic nerve and tract (mostly crossed in the rabbit chiasma), the dorsal nucleus of the lateral geniculate body, the optic radiation, a sequence of cellular elements situated in the layers of the cortex, and finally the corticifugal fibers returning from the cortex through the optic radiation. The preparation and manipulation of the animals have been described in previous publications (Bartley and Bishop, 1933).

Most physiological recording from the cortex has dealt with its delimitation into cyto-architectonic fields. The response of any one field or loca-

[1] Aided by a grant (to G. H. Bishop) for research in neurophysiology from the Rockefeller Foundation.

tion must be extremely complex, and its record must be the sum of many different responses of elements in different layers or positions in the cortex. It may be expected, from the degree of anatomical stratification exhibited, that a vertical differentiation should be quite as specific as a horizontal one, although, as emphasized by Lorente de Nó (1934a), the too obvious interpretation of the stratification of the cortex may lead to a deceptively simple concept of the functional relationships of the elements concerned. To the extent, however, that one stratum contains elements that are alike, and to the extent that they can be *activated synchronously*, such a sheet of elements will be comparable to a segment of peripheral nerve occupied by a volley of impulses. Electrodes in contact with this stratum, above and below a given level, need not be in contact with the same identical elements to give a valid record of such elements, but need only be in contact with similar elements active at the same time.

TECHNIC. In general, only those records are available for analysis in which the response to stimulation is large relative to the spontaneous activity occurring. The usual cortical responses of rabbits under light ether anesthesia are of the order of one millivolt or more, but significant elements of the responses may be only one-tenth of this, and less than the level of spontaneous cortical oscillations. Maximal single stimuli have usually been employed, at intervals of one second, two or more series of several successive shocks each being delivered for each strip of record. Electrodes are placed on the surface, and at various depths in the cortex, optic radiation, etc., and different pairs employed subtending different strata. The stimulating electrodes are not grounded, the rabbit rests on an insulated table, the single ground connection is usually the deeper of two electrodes, and negativity at this electrode gives an upward deflection in the record. After each experiment the carotid is injected toward the head with alcohol-bichloride fixative, the veins opened, the brain then removed to fixative and the significant parts finally sectioned and stained to locate the electrode positions with respect to the cortical structure. Usually a small blood clot locates the path of a needle electrode, otherwise tissue distortion is found. The surface electrodes consist of fine steel sewing needles (no. 10) with points filed off, the ends embedded in a block of celluloid cement 2 to 3 mm. in diameter whose flat surface penetrated by the needle distributes its weight. The deep electrodes are similar needles with points, coated with insulating cement to within 1 mm. of the tip. They are thrust as tangentially as convenient to the desired depths, and are unsupported except by their contact with the cortex, being free to move with the movements of the exposed brain accompanying respiration. Fine flexible wires connect each electrode to corresponding points of each of two selector switches, one of which leads to the ground of the amplifier, the other to the grid. Records are taken on 60 mm. bromide paper, drawn across and in

contact with the face of the oscillograph, whose beam moves only vertically. Another oscillograph in parallel to this but with time axis deflection presents the record visually. The start of this time deflection causes a disturbance that is recorded as a shock-like deflection preceding the stimulus artefact, which marks 1-second periods on the record.

Considerations of interpretation. Several general propositions may be applied to the cortex treated from the point of view of the physiology of peripheral nerve trunks. 1. Insofar as elements of a given type (for instance, cells with short processes) lie effectively between such electrodes, it will not matter whether the electrodes are close to these elements or at some distance above and below, or whether exactly vertically over each other; such structures will behave as a sheet of elements, or a polarized layer, imposing whatever difference of potential they set up in their ac-

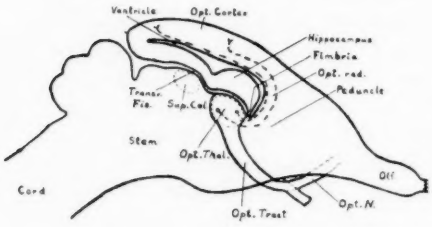

Fig. 1

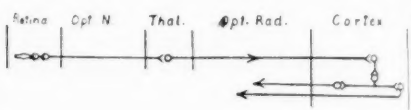

Fig. 2

Fig. 1. Diagrammatic projection of the optic pathway of the rabbit on a sagittal plane.

Fig. 2. Diagram of the optic pathway to compare with a linear stretch of peripheral nerve. Electrodes placed one above the other in the cortex will record impulses traveling in both directions, with complications of synapses between leads, etc., as discussed in text.

tivity on all the tissues adjacent. 2. Insofar, however, as such elements extend vertically through the cortex, the distance between leads along such structures will affect the diphasic record quite as in peripheral nerve trunks, and the amplitude and form as recorded will be a function of such separation of leads. 3. Further, if an element originates between leads, passing only the second, it will tend to give a monphasic (second phase) component of potential instead of a diphasic one; if it passes the first lead and terminates between leads, mainly a first phase will appear. For instance, since the number of cell axons leaving the cortex increases with depth in the cortex, the lower of two electrodes in the cortex will be in contact with a greater density of such axons than the upper, by the number which arise between electrodes, and with conduction downward in the cortex, the "second phase" of the summed diphasic potential assignable to them will be greater than the first. If the summation is such that individual responses

are not recognizable, but only the mass effect is recorded, the upper electrode will then be on the average less negative, that is, more positive, than the lower, and the net effect of conduction through such elements in the cortex may be a surface-positive potential wave, more or less monophasic, such as is usually found in cortical records. Finally, 4, since elements, or portions of elements lying parallel to the surface of the cortex, will in general run in random directions, their potentials will tend to annul each other, and only the vertical components of the potentials of such elements will affect the record. Any cortical record must therefore be an incomplete one.

The pathway of the visual impulse. To facilitate the consideration of the optic pathway as a modified peripheral nerve trunk, a diagrammatic sketch of its course may be presented (figs. 1 and 2). The fibers of the optic radiation pass downward and forward from the thalamus, in an arc, then upward and posteriorly to the optic area. The region from which responses to optic nerve stimulation can be obtained is roughly oval. It extends from the posterior pole of the cortex for 12 to 15 mm., or half way to the olfactory bulb, and from slightly medial to a shallow sulcus (which lies, in the rabbit, about 4 mm. from and parallel to the midline), it extends laterally 10 to 12 mm. It corresponds fairly well with the area striata as defined by Rose (1931) except that the area we find responsive may overlap what Rose pictures as the parastriate; we find the active-inactive margin to vary by 2 mm. or more from rabbit to rabbit.

The afferent fibers entering the cortex, at least in the cat and man, do not pass completely through it, but terminate in the region of the internal granular layer, roughly in the middle of the cortex, corresponding to the stria of Gennari of such animals as show this definitely (Cajal, 1923; Poliak, 1932). The first cortical neurones to respond should not therefore have their synapses located above this level. The granular layer is a region of cells with axis cylinders which pass both upward and downward in the cortex, but many of which do not leave the cortex. Both above and below the internal granular layer are regions of pyramid cells (superficial and deep) whose axons run in general perpendicularly through the cortex toward the basal white matter. The impulse started in the optic nerve must therefore pass in general to the middle layers in a direction toward the surface of the cortex, then, after synapsing one or more times, proceed (at least in part) further toward the surface, and finally reverse in direction after further synapses and pass from surface to the interior.

The activities in these successive components of the pathway may be expected to be set off from each other, and to be therefore recognizable, by temporal delays at synapses, by differences in amplitude as recorded from different depths in the cortex, and possibly by polarity of the potential record. Further, as the mass impulse, starting as a synchronous activity in parallel axons of the optic nerve, passes along fibers having different

conduction rates, and across synapses having different accessibilities, the individual elements of the response will be progressively dispersed, the synchronous volley be modified to a scattered distribution in which the individual responses will overlap and be summated to form a longer-lasting potential elevation. The form of the earlier waves, less dispersed temporally, will tend to be determined by the form of the individual impulse in each similar element. The form of the later responses will be a function largely of the *temporal distribution* of the responses of the individual elements. Finally, if at some region a single response in each element sets up repetitive responses in following elements, the temporal distribution of responses, and thus the form of their summated effect, will be still further modified. The records of cortical potentials may be examined from the point of view of these propositions.

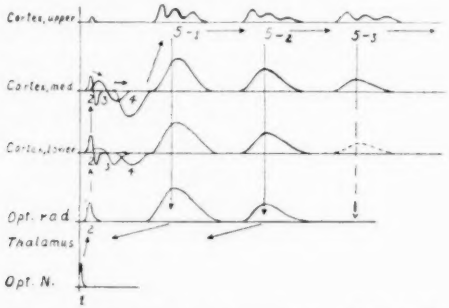

Fig. 3. Diagram of responses of the optic pathway. At least four elements of the response, following the response of the optic nerve, can be distinguished in some records, although any two adjacent elements, each presumably complex, may be confluent in a given record. The last of these four may be repeated several times at about $\frac{1}{5}$ second intervals following a single shock. There is a discharge of corticofugal fibers during at least the first of these repetitive cortical discharges, which appears to facilitate the thalamic neurones to a second discharge from the optic nerve.

Form of cortical response. The first potential observable following a single strong shock applied to the optic nerve (fig. 3, *2*) is a narrow spike, typically diphasic, followed immediately by a second somewhat broader diphasic deflection (*3*). These two may be fused, to form a single diphasic wave, the first phase of which often passes off the record at a sensitivity suitable for recording subsequent events, or they may be fairly well separated from one another. The first of these must include whatever is recorded of the response of afferent fibers from the thalamus, and may also include responses of cortical elements. Following these occurs a long-lasting, surface-negative deflection (*4*), followed in turn by a long-lasting surface-positive deflection. The latter is usually the most prominent

feature of the cycle (5-1); it will be referred to as the main cortical response. It is accompanied by a discharge of corticofugal fibers in the optic radiation, and may be repeated (5-2, etc.) at about $\frac{1}{5}$ second intervals, but with decreasing amplitude, until it finally becomes undistinguishable above the spontaneous activity. The potentials between the first response and the main response are recorded quite differently from different depths of the cortex. In previous papers (Bartley and Bishop, 1933a, b) we have interpreted this main cortical response as the first repetition of the deflection immediately following the shock artefact. It is now obvious that it is one of a progressive series of events initiated by the first response, but not a duplicate of it, and is itself the first of the repetitive series. For reasons that will appear below we cannot say at present whether the events preceding this main discharge are also repeated, but they probably are.

These are the responses so far differentiated, and we do not suppose that they are simple. Each may be the summation of the responses of several different varieties of neurones. Their time relations are as follows, varying considerably in different animals, with the strength of stimulation, and with other conditions conveniently disposed of as the "state of the animal." The initial discharge commences as early as 3 to 5 ms. after the shock, its duration is uncertain because it is diphasic, but is short enough to suggest that each element responds only once to each shock, that is, the relation of optic nerve fiber response to optic radiation fiber response is one to one across the thalamic synapse (for single stimuli). The next cortical response commences either during the first phase of the initial discharge, or early in its second phase, 10 to 20 ms. after the shock. It also is diphasic, and is sometimes brief enough, allowing for temporal dispersion at synapses, to suggest that here also a one to one relation exists across the synapses. The negative deflection is much longer in duration; its start is usually confluent with the second phase of the previous potential, its duration 50 to 100 ms. It might represent either a summated and asynchronous repetitive discharge, a series of overlapping discharges in successive elements, or a "slow potential" in each element responding. The main surface-positive elevation, which is accompanied by the summed activity of corticofugal fibers in the optic radiation, may arise smoothly from the preceding negative wave, or be apparently separated from it; its start is difficult to measure, but its crest falls typically at about 180 ms. after the shock, and is repeated once or more, often with strikingly similar configuration, at $\frac{1}{5}$ to $\frac{1}{3}$ second intervals thereafter. Its duration may vary from 80 to 200 ms.

Both the form and the magnitude of each component of potential in a given animal are functions of the depth in the cortex from which the potentials are led off. From two electrodes, one on the surface and a second above the internal granular layer (in the superficial pyramids), the initial

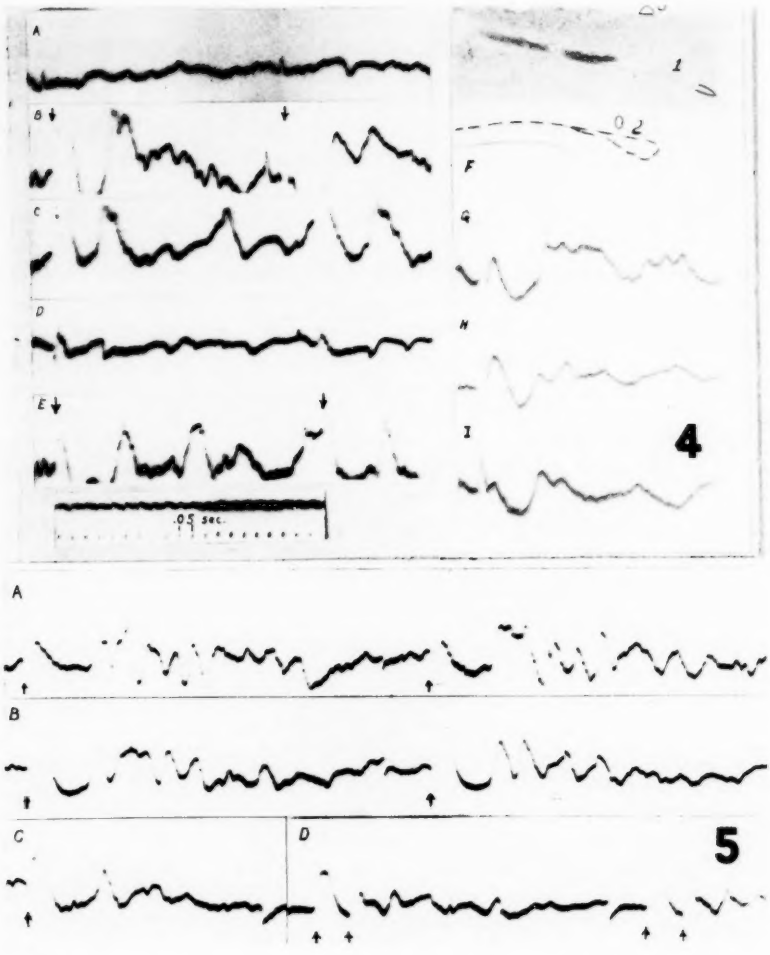

Fig. 4. Cortical records from electrodes whose position is shown in *F*, a section passing through the trace of needle *1*. The circle locating the point of needle *2* is projected from another section. While *2* appears to be definitely below *1*, it ran at a sharper angle, and its uninsulated point made contact with the cells above the fiber layer. Needles *1* and *2* are therefore effectively at the upper and lower margins of the sixth stratum of the cortex. *A*, spontaneous activity; *B*, leads *3* to *1*; *C*, lead *3* to *2*; *D*, leads *1* to *2*; *E*, *1* to *2* at three times the sensitivity of recording apparatus. A second stimulus falls in a repetitive response to the first. *G*, *H*, *I*, responses to the first, second and eleventh stimuli of a series at 1 per second, leads at surface and below cortex. Same preparation as above, but after more than an hour's use. Each

discharge appears as a low, sometimes nearly monophasic, surface-positive spike. Led from electrodes deeper in the cortex, it is higher and distinctly diphasic, the first phase surface-positive (fig. 4, *H*). That is, the proximal (or ground, deep) electrode is first negative; the impulse is traveling toward the cortex surface; and most of the elements presumably end before they reach the surface. The cortical response following this is not usually recorded by leads subtending only the surface layers, and from deeper electrodes, its first phase seems to be more prominent from the shallower, its second from the deeper strata. Its origin would seem to be from elements in the internal granular and deep pyramidal layers. It is recorded not only from localized leads, but also when one lead is on the surface and the second at an indifferent point,[2] either below the cortex or elsewhere on the head (fig. 4, *G-I*).

The surface-negative wave following this is likewise absent or weak in superficial leads, present in deep leads; and this is recorded from leads across the whole cortex, but less sharply than from the middle layers. The main surface-positive wave is recorded at every level and from the optic radiation. It presumably includes the discharge of pyramidal cells, both superficial and deep. At any depth, and with any separation of electrodes, the deeper electrode is typically negative to the shallower, the

strip 1 second. The first two waves are well separated out, the third (downward deflection) is confluent with the second phase of the second. The fourth wave, at least in *G*, is set off by its abrupt rise from the third, and is repeated with characteristic form. Note falling off in amplitude and in complexity of the elevations with repetition, even at 1 per second.

Fig. 5. *A* and *B*, records of responses, 2 shocks each, at 1 second interval, at two different regions of the cortex. Electrodes at surface and below the cortex. Except for the shock distortion, which interferes with the first part of the record, successive responses of the same area vary as much as responses from two different regions. *C*, a record taken after some time, similar leads to *A* and *B*, showing simplification to a definite 5-per-second rhythm of a picture previously much more complex. *D*, two pairs of shocks, the second of each following the first by about 140 ms., and giving a smaller response whose waves alternate with those of the first shocks. That is, the first wave following the second shock is the main response to the first shock (compare *C*), the next wave is the main response to the second shock, etc.

[2] In a technical sense, to an impulse activating the whole optic cortex synchronously, no electrode is an *indifferent* one. Such an electrode, for instance, on the side of the head, is effectually in contact, through head and brain structures acting as an extension of that electrode, with the sheet of fibers composing the optic radiation underlying the optic cortex. The optic cortex is large enough in area so that, between an electrode on its surface, except near the margin, and one anywhere on the head, no significant shunting takes place. In confirmation of this view, there is no significant difference in the record of response to stimulation wherever the second electrode is placed, until it enters the optic cortex itself (or the optic radiation leading to it).

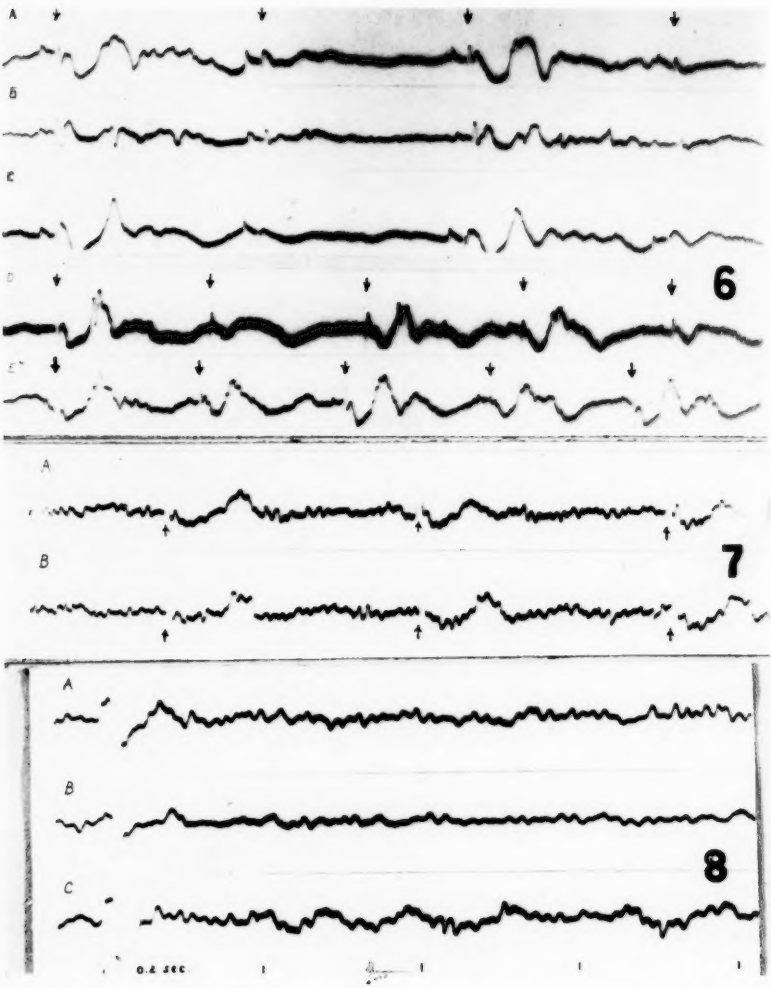

Fig. 6. Responses of the optic cortex to shocks at 1 second intervals applied to the optic nerve. With medium strength shocks (below maximal) only every alternate stimulus is effective (*A* to *C*). With stronger shocks, every one takes effect, but with alternations in amplitude (*E*). Still stronger shocks will in some rabbits each be equally effective. Note that when the shock is ineffective in causing a cortical response, even the first element of the response fails to occur. In *D*, at a strength between *A* and *E*, the second response fails to occur, but the fourth is present, the fifth being low.

A, one lead electrode on cortical surface, the second a needle whose uninsulated point lay below cortex. *B*, surface to granular layer, about middle of cortex. *C*,

amplitude of the wave being roughly a function of the distance between electrodes. Its configuration varies, sometimes having a smooth sine form, oftener consisting of a long elevation with ripples or definite peaks superposed on it, a common form being one main spike rising abruptly from the negative deflection, followed by a succession of 1 to 4 lesser spikes. Recorded from superficial layers it may consist only of such a series of apparently monophasic spikes, while from lower strata of the same preparation the spikes are nearly absent and only a long smooth elevation appears.

It is not uncommon for this elevation to vary during an experiment, although at any one time it is remarkably constant over the whole optic cortex except close to the margins. Here the spike-like prominences may tend to disappear, and the whole elevation may fail to repeat at $\frac{1}{5}$ second intervals, while still repeating in the central area. In other animals only a series of low monophasic or diphasic spikes is recorded from the periphery of the optic area. As the preparation ages, even the central areas show less repetition and less prominent spikes. This 5-per-second repetition is not always obvious in more complicated records, where the breaking up of the discharge into separate spikes may result in a nearly continuous series of spikes lasting for a second or more (fig. 5). In such cases, however, the first interval, between the shock and the main discharge, is of the usual duration and form; and as the preparation ages, the complexities tend to drop out, and the individual spikes to segregate into 5-per-second groups, which finally fuse to broad and relatively smooth waves (fig. 5, *C*). The course of such a transformation suggests that without an anesthetic, and in the unopened skull, the complexity of cortical activity would be such as to defeat analysis. Often, as has been described previously (Bartley and

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granular layer to below cortex. *D* and *E*, surface to below cortex. The negative deflection preceding the main positive response is greater in the lower half of the cortex, but present in records from across the whole cortex.

Fig. 7. *A*, shocks to optic nerve, 1 per second, response of cortex, below maximal stimulation. Note that time between shock and main response *decreases* with repetition, although the response becomes smaller. *B*, same with stronger shocks. The time for the first response is about the same as in *A*, but the times for subsequent responses decrease less than in *A*, although the responses are larger. With still weaker shocks than in *A*, even the first response may have a shortened delay.

Fig. 8. Responses of the cortex to brief flashes of light on the retina. Three successive flashes at 1.8 second intervals. Diphasic response followed by a monophasic, similar to response to shocks on optic nerve. The interval between start of diphasic and monophasic responses is less than usual, but the occasional spontaneous rhythm appearing, shown in *C*, is also of higher frequency, being in this rabbit 7 per second. The interval between the shock and the first main response is usually somewhat less than between subsequent repetitions; in the case of electrical stimulation, typically 160 to 180 ms. shock to maximum of main response, 200 ms. between successive maxima.

Bishop, *l.c.*), one of a series of such repetitive elevations will reverse in sign, either partially, appearing as a diphasic or triphasic elevation, or nearly completely. Sometimes this may be interpreted as the result of interference by a large spontaneous wave; at other times the phenomenon is repeated at the same place in the cycle with successive stimuli. This occurrence has been too haphazard to permit of adequate investigation.

The above picture is a composite one, both with respect to time relations and form, and its details vary from case to case. For instance, the first two elements are often completely fused; but their separation in some cases with different forms serves to distinguish them as different events. One further fact is of significance in this connection. When maximal stimuli are delivered, even so infrequently as one per second, the response to the second is decidedly less in amplitude than to the first, and successive responses may decrease further. The shorter the interval between stimuli, for maximal shocks, the greater the decrease in responses after the first. When such responses are decreased, however, usually *all components* decrease, beginning with the initial discharge. That is, the cortex may follow whatever is delivered to it, other evidence indicating that the refractoriness demonstrable is at the thalamic synapses (fig. 6).

Evidence has been presented previously suggesting that the thalamus determined the rhythmic activity of the cortex, even the spontaneous activity, as well as the rhythmic alteration of excitability exhibited to submaximal shocks (Bishop, 1933). We now have further evidence (to be presented later) that a return circuit from cortex to thalamus is involved in conditioning the thalamus to a second stimulus from the optic nerve, and thus affecting, perhaps determining, the inherent rhythm of the whole pathway. Dusser de Barenne (1933) has emphasized the intimate relations between cortical and thalamic functioning as evidenced by strychninization. Our experiments seem to indicate that an impulse arriving at the cortex sets up an impulse that returns to the thalamus, determining its response, and through it, the response of the cortex to a second stimulus. A similar circuit is involved in spontaneous activity, which will account for the 5-per-second rhythm that both spontaneous and induced activities exhibit. That is, the time required for the impulse to make this circuit is about $\frac{1}{5}$ second; most of this time is spent in traversing the cortex; the various events described above are some indication of how the impulse is spending this time; and the return discharge to the thalamus takes place at the time of the main surface-positive wave. The natural inference is that successive repetitions of this elevation signalize successive passages of this circuit by the impulse, which, however, finally either dies away, or its elements become temporally dispersed, or both, or it merges into the spontaneous rhythm already present.

Relation of form of potential to summation of unit responses. Of the four

events which make up the first cycle of the cortical response, it has been suggested above that the first two might be fairly simple summations of diphasic potentials. The next two are effectually monophasic in the record, and might represent summations of elements more temporally dispersed. The character of such possible summation may be dealt with somewhat as follows.

The summation of any diphasic potentials, provided the two phases of the units to be summed are equal in area, should be in turn a diphasic potential, with equal areas of the two phases. The form and amplitude of the summed potential will be functions of the time relations of the unit elements, particularly of their frequency. Where the time over which summation takes place is long compared to the duration of a single element of potential, the form of the summation will be chiefly a function of the frequency rather than of the form of the unit. Such a summation may be termed a *second order* potential.

The fact that the record led from the optic radiation during the main cortical discharge has the duration of this discharge indicates that the record from the radiation, which contains only nerve fibers, is a summation of *fiber* potentials. The record from the cortex itself, since this contains the same fibers, must be in part at least a similar summation, whatever component of "slow" potentials assignable to cell bodies, after-potentials, etc., it might also involve. This cortical potential is, however, comparable to slow potentials recorded from cell masses by other workers, and its analysis becomes of considerable interest. The leads from which it is recorded are ostensibly such as to record at least some of its fibers diphasically, yet its form is typically monophasic, and its polarity such as to indicate a predominance of the second phase in the record (negativity at the second electrode along the path of the impulse, *i.e.*, the deeper of two in the cortex). The question arises to what extent the form of this potential can be explained without recourse to the assumption of long-lasting unit potentials, that is, as the summation of typical fiber potentials such as peripheral nerve trunks exhibit.

It has been noted elsewhere (Bishop, 1935) that only a slight difference in the effective areas of the two phases of the unit potentials to be summed may be required, at certain frequency distributions, to give virtually completely monophasic summation potentials. It has been suggested above that in the cortex, more second phases will be recorded from the lower strata than first phases from the upper, in an efferent discharge from the cortex, which will amount to the same thing as a difference in areas of the two phases. Not only the areas of such summated potentials, but the times of their maxima, and even their apparent starts, will depend on the frequency of response of the units of which they are the summation, whether the frequency is a matter or repetition of response in each element, or tem-

poral dispersion of responses in different elements. The frequency distribution of response may be expected to vary in the cortical discharge for numerous reasons, and certain phenomena may perhaps be explained on this basis. For instance, when successive equal shocks at one second intervals are applied to the optic nerve, the first of the series usually gives the largest response, but subsequent responses may appear *earlier* after the shock (fig. 7). It might be expected that the same factors which cause the response to be *less* should also cause it to occur *later*. On the other hand, if a first shock is in fact more effective, one of its effects should be to accelerate more rapidly the frequency of the final discharge, whether by increasing (due to facilitation between one unit and another) the frequency of repetition in each unit, or by inducing closer synchronization of the discharges in parallel units. From the fact that the cortical potential under consideration is typically monophasic, one might infer that the initial acceleration of its elementary frequency is insufficient to cause a diphasic summation. An effectively stronger (first) stimulus should then tend to render the result diphasic, and its first effect should be to bring the apparent start, and even the apparent crest of the summation, *later*. The starts of cortical potentials are difficult to measure accurately in the presence of spontaneous oscillations. The crests often behave as the above scheme suggests.

If single shocks are applied, one actually stronger than the other, the situation is similar. The stronger (below maximal) gives the greater amplitude of the main cortical response, but this response may be later than that to a weaker shock, although the initial response of the cortex is earlier, the stronger the stimulus; that is, more time is *apparently* consumed by a strong impulse traversing the cortical pathway than by a weaker one. When such shocks are delivered successively, the later responses to the strong series occur about as early as the first response to the weak series. Again an apparently less effective impulse traverses the cortex in less time than a more effective one, unless the form of the summation curve gives a deceptive picture of the time course of the cortical response.

Similar considerations might be invoked to explain the variation in the prominence of the surface-negative wave that precedes the main discharge from the cortex; but our information about this is still too unsystematic to justify elaborate speculation. Another factor, however, may be mentioned to which the foregoing arguments might be applied. If the 5-per-second repetitions of the cortex are a function of a loop pathway from cortex to thalamus and back, the repetitive discharges should involve repetitions of *all* the cortical events appearing in a first cycle. This does not appear in the records, and it may be surmised that temporal dispersion, as a cause of overlapping of the components, as well as of a change of the frequency of their units, will account for the apparent loss of all but the most prominent component. The same sort of thing should occur even in the case of the

first cycle, in the case of response to stimulation of the retina by brief flashes of light. Here the retina itself is known to give to the optic nerve not a single synchronized volley of stimuli, but a temporally dispersed repetitive discharge in the fibers activated. The result (fig. 8) is a diphasic first potential at the cortex which may be inferred to include both the afferent fiber discharge and the first cortical discharges. This is followed by a monophasic surface-positive wave comparable to the main cortical discharge. Occasionally a repetition of the latter can be detected, as in the case of direct stimulation of the optic nerve, but this is unusual, as could be expected on the basis of the arguments presented, in view of the dispersed retinal discharge of which it is the consequence.

While the cortical response to stimulation has a form that is reasonable as a summation of *fiber* potentials, it would be hazardous to infer that only fiber potentials contributed to it. It should be noted that leads from across the cortex, which is less than 3 mm. deep, compare to peripheral nerve in contact with which the recording electrodes are separated by only 2 to 3 mm. If conduction is 50 m.p.s., the interval between arrivals at the two electrodes would be 0.05 ms., or $\frac{1}{4}$ the rising phase of the action potential. This would result in an insignificant contribution of time-potential area per impulse as compared to a monophasic record, yet the responses of the cortex can be over 1 mv. in amplitude, and 100 ms. in duration. This diphasic suppression would be obviated to some unknown extent by the partially monophasic recording of part of the elements of the cortex, those arising effectively between leads. One could assume also that in the 100 ms. which the main cortical response occupies, a given element could act repetitively up to, say, 50 responses.

Discussion. The foregoing experiments concern the responses of the optic cortex to stimulation of the pathway of the visual impulse. The phenomena have been discussed as if the responses were visual responses. It can only be claimed at present that they are physiological responses correlated with stimuli potentially visual. We do not know where "vision" is consummated, but the optic cortex response must be a preliminary to it. The question arises whether the cortex responds to single shocks to the optic nerve in a manner similar to its response in vision.

Two differences can be pointed out at once. First, facilitation is readily demonstrated below the level of the cortex, and the difference between the single volley sent up to this level after a single shock, and the scattered repetitive discharge of the optic nerve to retinal stimulation by light must make a difference in the organization of the response at higher levels. Presumably the same cortical elements respond in the two cases, in roughly the same sequence, but possibly with much more intimate mutual interaction in the more complicated situation. Secondly, the long "refractoriness" demonstrable after a maximal shock obviously does not apply directly in

vision, unless we compare such a shock to a blinding flash of light. To less than maximal stimulation, it has been shown (Bartley, 1936) that elements not activated by the first shock can be activated by a second shock of the same strength at any time after the first, owing to the fact that such elements become accessible to stimulation rhythmically, but usually more or less out of phase with each other. A given unit locus (in the retina) must therefore be able to employ any one of a number of elements, in projecting itself upon the cortex, and to employ them alternately, to accomplish "continuous" vision. The variations in responses we obtain may then be assigned in part to a variable number of elements receptive at a given instant, starting from a condition of "rest."

In the rabbit, the presence of the 5-per-second rhythm, corresponding presumably to the 10-per-second rhythm recorded from the occiput of many human subjects, apparently signalizes a rhythm of excitability of the optic pathway. Quite as this rhythm is broken up or decreased in the human case by light stimuli, so both the rhythmic spontaneous responses and the underlying excitability rhythm which they connote can be altered by stimulation of the optic nerve in rabbits. In the human case, the light stimulus presumably scatters the synchronism of elements whose previous summation amounts to a potential large enough to be recorded. In the rabbit, a single volley of impulses over the optic nerve resynchronizes the elements to an even more pronounced rhythm, or if the rhythm is already maximal, such a volley may be ineffective if it falls in the surface-negative inexcitable phase of the spontaneous rhythm. It would appear then that an alert or continuously receptive state is characterized by an asynchronism of the elements involved, and that in a condition of rest (lack of stimulation) such synchronization tends to be reëstablished, possibly by mutual facilitation of parallel spontaneously rhythmic elements. The first part of a persistent stimulus to the eye should then be expended in reorganizing the state of "rest," and this may be a factor involved in focusing the attention on the stimulus, a process that notoriously requires time.

The spontaneous rhythm exhibited, as well as part of the response to stimulation, may then be assigned to coördinating circuits whose function is to facilitate the visual pathway, but whose activity alone does not involve vision. Presumably another part of the response to stimulation, whose magnitude is conditioned by these coördinating circuits, is involved in transmitting the visual impulse itself. In accordance with this, a response to stimulation can always be obtained which is much greater in amplitude than any spontaneous activity. The mode of action indicated here involves something like the reverberating circuits for which Lorente de Nó (1934b) has found histological evidence in the cortex.

Certain differences between responses to light on the retina and to shocks across the optic nerve are therefore assignable primarily to differences in

the organization of the impulse at the level of the thalamus and above, and aside from this, the cortex appears to respond to each in accordance with the same essential characteristics. The record is simpler in appearance and larger in amplitude when the elements responding are as much as possible summated, and the record disappears when, under continuous stimulation by light, the responses of individual elements are completely asynchronous, only the "on" and "off" effects appearing in the cortical record. Single shocks to the optic nerve then represent the opposite extreme to continuous stimulation of the retina by light, so far as the cortical response is concerned, and enough intermediate stages are becoming known, through repetitive shocks on the one hand or repetitive short flashes of light on the other, to effectively bridge the gap between them, and allow each to be interpreted in terms of the other. It thus appears that the more complicated the stimulus, the more simple is the *record* of the response. It is reasonable to infer that the visual act is not accomplished without each chain of elements comprising a unit pathway from retina to brain being occupied by the activity of which the cortical record to a single shock is an index, even though an incomplete one. This record differs from the record of a response to retinal stimulation chiefly in that the latter is the more confused picture, that is, it is the more complex in the temporal relations of its elements.

SUMMARY

A single maximal stimulus applied to the fibers of the optic nerve results in a response of the optic cortex of which four components can be differentiated. The first two are diphasic potentials, often fused to one, of which the first phases involve positivity of the surface of the cortex. The third is a slow surface-negative deflection, the fourth a slow surface-positive deflection, the latter often having superposed upon it a series of monophasic or diphasic spikes. When smooth, it perhaps consists of the smooth summation of such spikes temporally dispersed. The fourth component at least is typically repeated at about $\frac{1}{3}$ second intervals during one second or less.

These components, any two of which may be confluent in a given record, are differentiated by their differences of form when separate, by their differential changes under manipulation, and by the fact that they are recorded differently when led from different levels in the vertical stratification of the cortex.

Considerations of the relation of the form of unit potentials (potentials of unit neural elements) to the form of the summated response, indicate that the form of the summated wave will be a function of the degree of diphasicity of the unit elements as led off, of the spatial relations of the units to the electrodes, and of the acceleration of the frequency of unit

responses, aside from the mere numbers of such units activated by a given stimulus.

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ABSORPTION OF SODIUM CHLORIDE FROM THE SMALL INTESTINE AT VARIOUS DEGREES OF ANOXEMIA[1]

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In a recent paper the effect of anoxemia on absorption of water from the small intestine was reported by Van Liere, David and Lough (1936). In this paper it was shown that absorption of water takes place much more readily at rather pronounced low oxygen tensions than at normal atmospheric pressure. In view of these findings it was thought worth while to study the absorption of an electrolyte from the gut under anoxemic conditions.

Hamburger (1896) found that absorption of salt solutions from the intestines of dogs which had been dead from one to twenty-four hours proceeded in the same manner as living dogs. Magee and Macleod (1929) reported that the walls of segments of gut which had been devitalized became more permeable to solutions and electrolytes than normal gut. One might deduce from these experiments that oxygen is not absolutely necessary for absorption of electrolytes from the intestine.

METHODS. With slight variations the same methods were used as those reported in the paper already mentioned. Barbitalized dogs and cats which had been starved twenty-four hours previous to the experiment were used. Two animals were chosen which were as near the same weight and age as it was possible to obtain them. In a number of experiments litter mates were used. One animal was used as a control and the other subjected to anoxemia.

A midline incision was made in the abdominal wall and the small intestines exposed. Practically the entire small intestine with the exception of the duodenum was used for a loop. The loops of the two animals were made of equal length by actual measurement. They were washed out with 5 per cent glucose solution; care was exercised to recover all the fluid which had been used for washing.

A measured amount of 0.9 per cent sodium chloride solution was placed in each loop. Distention was avoided. The intestines were replaced in

[1] The expenses of this investigation were defrayed by a grant from the Committee on Scientific Investigation of the American Medical Association.

the abdominal cavity and the wound closed with sutures. The animal to be subjected to anoxemia was placed in a steel respiratory chamber pre-

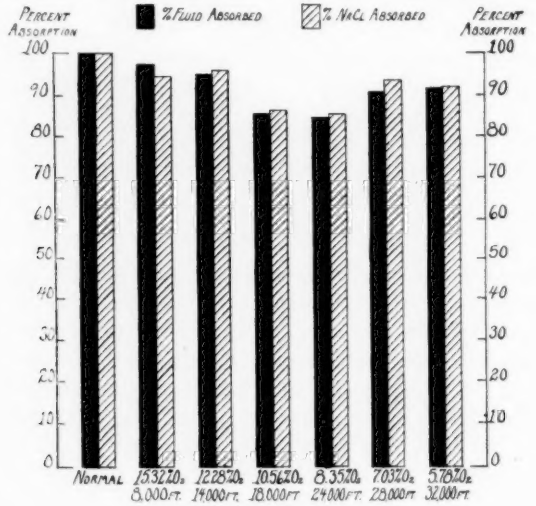

Fig. 1

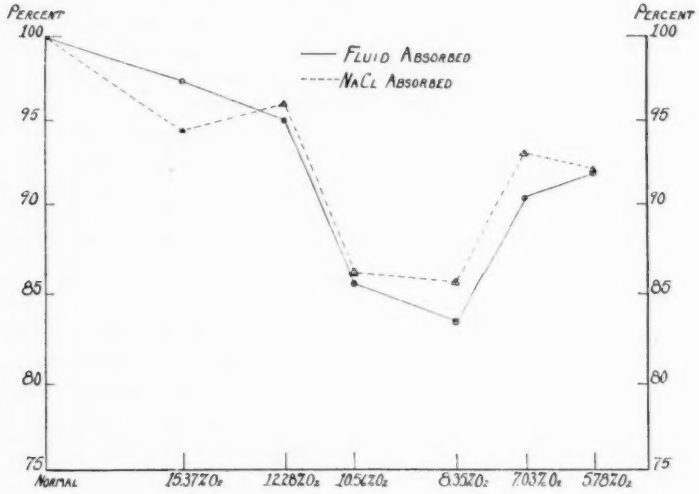

Fig. 2

viously described (Van Liere, 1927). The one which was to serve as a control was kept at atmospheric pressure. The salt solution was left in the intestinal loops for 40 minutes. The loops were then removed from the

body of the animal, slit open and the contents carefully measured. The actual amount of sodium chloride in the recovered fluid was determined by Van Slyke's modification of the Vollhard method.

The effect of the following percentages of oxygen on the absorption of salt solution from the small intestine was determined: 15.32, 12.28, 10.56, 8.35, 7.03 and 5.78.

Results. The accompanying graph and chart show the results obtained. It will be seen that anoxemia curtailed the amount of fluid and sodium chloride absorption. Anoxemia was most effective in decreasing the amount of absorption at oxygen percentages of 10.56 and 8.35. Lower percentages of oxygen (7.03 and 5.78) did not decrease the amount of absorption as much as did the oxygen percentages formerly mentioned. The fluid absorption and actual sodium chloride absorption practically ran parallel.

Discussion. The results reported here are quite different from those reported in a previous paper in which the effect of anoxemia on water absorption from the small intestine was studied. In the latter study it was reported that 15.37 per cent oxygen had no appreciable effect on the absorption of water from the small intestine, but that there was a decrease in the amount of absorption at 12.28 per cent oxygen. At higher degrees of anoxemia, however, absorption took place more readily from the small intestine in the anoxemic animals than in the controls.

The absorption of 0.9 per cent sodium chloride solution was diminished at all degrees of anoxemia. A significant reduction in the amount of absorption took place even at 15.32 per cent oxygen. This is a very mild degree of anoxemia and corresponds to an altitude of about 8000 feet. Many people live at such altitudes.

It is difficult to explain the fact that severe degrees of anoxemia are less effective in reducing the amount of absorption of 0.9 per cent sodium chloride from the small intestine than more moderate grades of anoxemia. It must be stated here that 126 animals were used in these experiments and with such a large series we feel that our results are fairly accurate. It may be that extremely low oxygen tensions actually injure the cells in the intestine and this may make them more permeable to the passage of the sodium chloride molecule. The lowest oxygen percentage used was 5.78 as it was found that the majority of barbitalized animals could not withstand lower oxygens tensions than this for the time necessary to perform the experiments.

It is hardly proper to compare the results reported in this paper with those obtained by Hamburger or those of Magee and Macleod. These authors worked with dead membranes and it is quite likely that dead membranes have a different permeability than do living ones.

The question of the reaction of the blood during anoxemia, and its effect

on absorption of water has been discussed at some length in a preceding paper (Van Liere, David and Lough). It is, of course, well known that the more severe the degree of anoxemia the lower the pH of the blood becomes. As the graph and chart show, however, there is no direct ratio between the amount of absorption of 0.9 per cent sodium chloride solution and the degree of anoxemia. The reaction of the blood, therefore, cannot be used to explain the reason for severe degrees of anoxemia being less effective in reducing the amount of absorption than more moderate degrees.

SUMMARY AND CONCLUSIONS

In studying the effect of various degrees of anoxemia on the absorption of 0.9 per cent sodium chloride solution from the small intestine of barbitalized dogs and cats it was found that low oxygen tensions decreased the amount of absorption. Even a mild degree of anoxemia (15.32 per cent oxygen) had a significant effect on absorption. The fluid absorption and the actual sodium chloride absorption practically ran parallel.

The greatest decrease of absorption of 0.9 per cent sodium chloride solution from the small intestine occurred at 10.56 and 8.35 per cent of oxygen. At more severe degrees of anoxemia (7.03 and 5.78 per cent of oxygen) absorption was not decreased as much as in the two ranges previously mentioned. It is suggested that extremely low oxygen tensions actually injure the cells of the intestinal membrane and that permeability is increased in this manner.

The conclusion which may be drawn from this work is that oxygen aids in the absorption of 0.9 per cent sodium chloride from the small intestine of the mammal.

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TRANSPLANTATION OF SINO-ATRIUM TO CONUS IN THE EMBRYONIC HEART IN VITRO

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The production of an ectopic pace-maker by transplantation of the sino-auricular node in adult hearts (7) leads to the question as to what would occur following a similar procedure in tubular embryonic hearts. Concerning the pace-maker in embryonic hearts no circumscribed mass of tissue comparable to a node can be distinguished. It is well established, however, that there exists a gradient of rhythmicity inclined from the venous to the arterial end. Furthermore the separation by section or ligature of sino-atrium from ventricle results in a difference in rate of beat of the two moieties, the venous portion contracting at a distinctly faster rate (2, 6, 3, 4, 5). It has also been shown by Paff (5) that the sino-atrial region dominates the remainder of the heart. Complete transection with accompanying functional disunion as manifested by a marked decrease in the rate of the ventricle is, under proper conditions, followed by reëstablishment of functional union. This is demonstrated by a marked "stepping up" of activity in the ventricle as new growth bridges the region of transection. With reëstablishment of a conductile pathway the beat in the two portions becomes synchronous again.

In the present group of experiments the author has transplanted the sino-atrium from the ventricle to the conus (cf. fig. 1) to determine whether or not it is possible for the pace-maker to dominate the ventriculo-conal region from this reversed location.

METHOD. Hearts were dissected from 55 hour chick embryos and washed in Tyrode solution. The sino-atrium was cut free from the ventricle and placed in apposition to the opposite end or the conus arteriosus (cf. fig. 1). The preparation was then very gently flooded with chicken blood plasma and mounted over a depression slide. Embryonic extract was not used.[1]

[1] In conducting these experiments it was felt that results would be more conclusive if each heart were preserved as a heart in the cultures. Too often in tissue cultivation the investigator is interested primarily in rapid growth. The companion to rapid growth is migration of cells. In preliminary experiments fresh embryonic extract and blood plasma were used. Results were discouraging. Growth was rapid and migration was extensive. In some cases the hearts lost their identity in twenty-four hours. Because of this, embryonic extract with its growth promoting substances was not used. Blood plasma supported sufficient growth and throughout the experimental period the hearts were identifiable as hearts rather than just pulsating masses of protoplasm.

Observations were made with the preparation in a warm chamber at 38°C. In taking rates of contraction the author's counts were confirmed by other observers. Eight successful experiments were obtained, and of this number data are presented below for four.

Observations. Following removal and sectioning of the hearts, the ventriculo-conal region beats at a distinctly lower rate than does the sino-atrium. Placement of the sino-atrium to the opposite end of the heart results at first in no alteration in this relationship. In a variable number of hours, however, striking changes become apparent. The two portions of the heart begin to grow together and then the behavior of the ventricle changes. The first expression of this is usually seen as a struggle to maintain its own rhythm against the opposing one of the sino-atrium. As growth in uniting tissue progresses, it is common to observe an apparent two to one rhythm between sino-atrial and ventriculo-conal regions. Within a few hours this relationship gives way to periods of synchronous

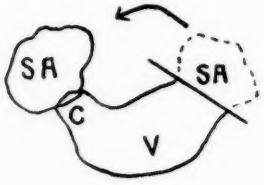

Fig. 1

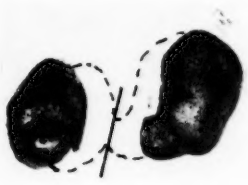

Fig. 2

Fig. 1. Heart 13 immediately after removal from embryo. Change of position of sino-atrium, *SA* from ventricle, *V*, to conus, *C*, indicated by arrow. Outline tracing x 28.

Fig. 2. Heart 13 after 27 hours in vitro. Tracing of heart at time when rhythm was synchronous superimposed on photograph taken after final observations. Plane of section indicated by solid line. Hematoxylin stain x 28.

activity during which the rate of the ventricle is increased to correspond to that of the sino-atrium. These periods of synchronicity alternate with ones during which the ventricle beats at a slower rate than the sino-atrium, sometimes in one to two relationship, sometimes quite independently. Finally a regular synchronous rhythm is established and persists.

After the establishment of a synchronous rhythm a significant change occurs when the hearts are transected in the region of new union between sino-atrium and conus (cf. fig. 2). Sectioning terminates the unified activity. Released from the sino-atrium, the ventriculo-conal region takes up its own rhythm, the rate falling distinctly below that of the sino-atrium. In the latter the rate is practically unchanged.

Fundamentally the behavior in each heart was the same. However, in certain particulars sufficient variation occurred as to make it advisable to present specific data for at least four of the eight successful experiments.

Heart 1. Hours after planting

1 HOUR	23 HOURS	25 HOURS	29 HOURS (CUT)
SA—♯186 VC— 69	SA—149 VC—126	SA and VC—142 (Synchronous)	SA—146 VC— 68

SA—Sino-atrium; VC—ventricle conus; ♯—number of beats per minute.

In heart 1, after 23 hours in vitro, periods of synchronicity alternated with periods of two to one activity. At 25 hours the beat was synchronous. This was difficult to decide at a temperature of 38°C. because the heart was beating so rapidly. By dropping the temperature several degrees the activity decreased sufficiently to permit of an unquestionable conclusion that the beat was synchronous. After sectioning at the place of union the rate of beat in the sino-atrium was changed but little (142 to 146 per minute). In the ventricle the decrease was marked (142 to 68 per minute).

Heart 13. Hours after planting

1 HOUR	19 HOURS	23 HOURS	27 HOURS (CUT)
SA—164 VC—120	SA and VC—94 (Synchronous)	SA and VC—84 (Synchronous)	SA—89 VC—62

In heart 13, when observed 17 hours after planting, a struggle seemed to be taking place between sino-atrium and ventriculo-conal region. For brief periods of variable duration they were synchronous. Within 2 hours (19 hrs.) a regular synchronous rhythm was established. Here again after cutting at 27 hours, the sino-atrium remained quite constant (84 to 89) but the ventricle and conus changed (84 to 62).

Heart 9. Hours after planting

1 HOUR	18 HOURS	21 HOURS	25 HOURS (CUT)
SA—180 VC—134	SA and VC—34 (Synchronous)	SA and VC—94 (Synchronous)	SA—96 VC—72

At 18 hours heart 9 was beating synchronous but the heart showed periods of activity alternating with periods of rest. This is a phenomenon which occurs frequently with prolonged cultivation and persists until transplantation to fresh medium. In this case, under uniform conditions, it gradually disappeared and three hours later the heart was again beating regularly. Note the decrease in rate of ventricle following sectioning at 25 hours.

Heart 8. Hours after planting

1 HOUR	20 HOURS	24 HOURS	28 HOURS (CUT)
SA—144 VC—122	SA and VC—96 (Synchronous)	SA and VC—115 (Synchronous)	SA—113 VC— 70

This preparation showed an unusually vigorous beat. Following synchronization, ventricular systole produced a decrease in diastolic diameter of fully 25 per cent. After cutting the region of union at 28 hours, the ventriculo-conal region was far less vigorous and its rate dropped from 115 to 70 per minute.

Discussion. Following the transplantation of the pace-maker and the production of a unified rhythm, the control of the sino-atrium over the ventriculo-conal region is proved by the fact that when the two are separated by transection the ventricle and conus again drop distinctly in rate of beat. This difference per minute in the four preparations presented was 78, 27, 24 and 43. In contrast to this, the sino-atrium maintains practically the same rate of beat as observed in the heart before transection (at the region of union). The question arises as to whether the ventricle and conus have been activated in the reverse direction. That this is so is proved by the fact that if, after a unified rhythm is established, the heart is made quiescent by cooling and then reactivated by warming, the sino-atrium beats first and is followed a split second later by the ventricle. As the rate increases each beat originates in the sino-atrium before the previous one is completed in the ventricle. Assuming the existence of a refractory period comparable in duration to that of the adult heart, the effective impulse is traversing the conus while the previous impulse is producing the wave-like systole of the ventricle.

It may also be significant that small masses of corpuscles within the lumen of the hearts are given an initial thrust toward the end of the ventricle opposite to the new sino-atrial location. In reference to this point Bremer (1) observed reversal of the beat in the heart of an embryo in situ, in which case blood was actually being pumped out of the veins. In his experiment the conus gained the ascendancy over the sino-atrium. Such a condition is exceptional but it leaves no doubt that reversal of the normal gradient in ventricle and conus is possible.

In establishing this new functional relationship between sino-atrium and ventricle certain conditions must be met. If union is to occur within twenty-four hours the cut surfaces of each moiety must be brought close together. Attempts to graft the sino-atrium on the intact lateral border of the conus met with no success in forty-eight hours of cultivation. Each portion of the heart contracted independently.

As a corollary to the above condition, myocardium must knit to myocardium. The embryonic muscle alone will furnish the anatomical basis for a functional pathway. In over a hundred experiments synchronization was never observed in sectioned hearts as the result of fusion of fibroblasts from the cut ends of the two parts.

CONCLUSIONS

1. The sino-atrium when transplanted to the conal end of the heart will, under proper conditions, dominate the ventriculo-conal region.

2. In the ventriculo-conal region activity is reversed.

3. The anatomical requirement is union of myocardium of sino-atrium to myocardium of the conus.

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INCREASED WATER EXCHANGE FOLLOWING ECK FISTULA IN DOGS

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Since Molitor and Pick (1) showed that water by mouth in dogs with Eck fistulae produces a greater blood dilution and a more prompt diuresis than occurs in normal animals, there have been numerous observations indicating that the liver plays a part in regulating the water content of the blood (2, 3, 4, 5, 6, 7, 8). It occurred to us that if a normal function of the liver is to retain a large proportion of ingested water and gradually release it to the blood, disturbance of this function, leading to more prompt elimination of water, should result in an earlier appearance of thirst and an increased intake and output over long periods of time. We have therefore studied the voluntary fluid intake and urinary excretion in dogs before and after the establishment of Eck fistulae.

METHODS. Normal dogs, kept in cages from which urine could be collected, were allowed access to water *ad lib.*, a 2 or 5 gallon crock being present in the cage at all times. The diet consisted of a mush made of bread and corn meal boiled with bones. The voluntary water intake and the urinary output were measured daily over a control period of at least 2 weeks. An Eck fistula was then established under Nembutal anesthesia, the animal allowed to recover from the immediate effects of the operation for 48 hours and observations on intake and output continued. As a further control, a dummy operation which consisted simply of stripping the portal vein and the vena cava as in preparation for an Eck fistula was performed on 2 dogs. This procedure was designed to produce as great an interference with the nerve supply of the liver as may be present in the Eck fistula animals. To further eliminate the possibility that destruction of a part of the nerve supply to the liver or other organs was responsible for the observed changes, intake and output were followed in 2 dogs before and after section of both splanchnic nerves. The influence of other procedures that damage the liver was observed in 2 dogs with ligation of the common bile duct and in 2 that were each given a one hour period of deep chloroform anesthesia.

Since not all dogs responded to the establishment of an Eck fistula with a markedly increased water exchange, it was of interest to correlate the

increase in intake and output with the degree of blood dilution that occurred after giving water by mouth. For this purpose we gave 20 cc. of water per kilo by stomach tube to 6 Eck fistula dogs on which intake and output were measured, and followed the change in the plasma chlorides at 20 minute intervals for 80 minutes. The maximum blood dilution taking place after this amount of water by mouth was determined on 10 normal animals, as was the average normal intake and output for the same season over a two week period. It is of incidental interest that a maximal dilution of the chlorides was produced by 20 cc. of water per kilo; 30 or 40 cc. per kilo was not always retained and in any case caused no greater decrease in the plasma chloride.

RESULTS. In table 1 are presented the daily voluntary water consumption of 8 dogs before and after the establishment of the Eck fistulae. Urinary output is not given, since it ran parallel to the water consumption,

TABLE 1

Effect of Eck fistula on the average daily water intake (figures represent averages of daily intakes for periods of one week)

DOG	PRE-OPERATIVE, WEEKS				POST-OPERATIVE, WEEKS							
	4	3	2	1	1	2	3	4	5	6	7	8
E-1		310	350	330	2,900	2,730	2,050	1,660	1,630	1,480		
E-2		371	230	270	450	290	245	420	370	230		
E-3			385	450	1,630	1,800	1,630	1,340	1,050	870	910	730
E-4	205	250	185	190	1,500	760	550	350	380	350	190	500
E-5			270	320	1,870	1,620	1,510	1,230	1,420	1,820	1,920	1,370
E-9			310	290	891	373	285	295				
E-10		180	184	166	671	340	285	295				
E-11			370	400	900	750	710	710	800			

which it commonly exceeded by 100 to 200 cc. The water taken in the food was not measured. The specific gravity of the urine was determined daily on a number of the animals; it was roughly in inverse proportion to the volume of urine excreted. The data on intake and output following the dummy operation, ligation of the common bile duct, splanchnic section, and administration of chloroform, are given in table 2. The only one of these procedures that appeared to affect water exchange was chloroform poisoning, which produced an increase comparable to that following an Eck fistula. Table 3 gives the correlation between voluntary intake and degree of chloride dilution following water by mouth in 6 Eck fistula dogs; data on chloride dilution and voluntary intake in 10 normal dogs are submitted for comparison. The correlation is surprisingly good. None of the Eck fistula animals that were at hand at the time the studies were made exhibited an average daily intake within the normal range, but the two

TABLE 2

Effect of splanchnicotomy, common bile duct ligation, stripping of veins, and chloroform on average daily intake

(S—splanchnicotomy, B—duct ligation, D—veins stripped, C—chloroform)

DOG	PRE-OPERATIVE, WEEKS			POST-OPERATIVE, WEEKS			
	3	2	1	1	2	3	4
S1		230	270	330	270	265	
S2		180	165	140	160	135	
B1		90	75	80	60	110	
B2		310	280	350	270	310	
D1	210	300	250	175	180	185	350
D2	425	400	370	250	260	320	240
C1		340	400	1,070	900	920	
C2		90	110	200	180	170	

TABLE 3

Correlation between voluntary daily intake and blood chloride dilution after water by mouth

A. Daily water intake per kilo in 10 normal dogs; average of determinations over a 2 week period

Average, 23 cc.
Maximum, 32 cc.
Minimum, 20 cc.

B. Per cent decrease of plasma chloride in 10 normal dogs after 20 cc. of water per kilo by mouth

Average, 2.8
Maximum, 5.0
Minimum, 1.2

C. Voluntary intake compared with per cent fall plasma chloride in Eck fistula dogs

DOG	PER CENT FALL CHLORIDE AFTER 20 CC. WATER PER KILO BY MOUTH	DAILY WATER INTAKE PER KILO; AVERAGE OF TWO WEEK PERIOD
E-6	5.0 4.2	55
E-7	9.0 7.8	73
E-8	7.0	46
E-9	2.7 2.5	37
E-10	1.5 3.0	34
E-11	7.5 6.3	57

(E-9 and E-10) that exceeded the normal intake by only a small margin showed a chloride dilution well within the limits found in normal dogs. In the remaining four, the increase in output is roughly proportional to the degree of chloride dilution. Maximum dilution was usually present at the 20 or 40 minute interval.

It should be noted that the subcutaneous injection of pituitrin had no effect on the increased intake and output of the Eck fistula dogs.

Discussion. The obvious explanation for the greatly increased water exchange that follows hepatic damage is based upon the same reasoning that induced us to perform these experiments. The greater blood dilution occurring after water by mouth in animals and patients with liver injury has been adequately confirmed (1, 2, 3, 4). According to Molitor and Pick (1) there is also a more prompt diuresis and a greater total excretion. Although little is known concerning the mechanism of thirst, it can be assumed that the prompt elimination of fluid would result in a more prompt reappearance of the desire for water.

Because the surgical procedures involved in making an Eck fistula probably produce some interference with the nerve supply of the liver, the control experiments of stripping the vena cava and portal vein (dummy Eck fistula) and sectioning both splanchnics were performed. Since neither of these procedures influenced the water exchange, while chloroform poisoning, involving no operative interference, did increase intake and output, we may assume that the change is produced by a direct suppression of the function of the parenchymatous tissue. While a high degree of liver injury also eventually occurs after ligation of the common bile duct, this procedure introduces digestive disturbances and other complicating factors. The pathological changes in the liver following duct ligation are known to be progressive, but our animals were studied for three weeks only because of the cachexia that developed.

The fact that the specific gravity of the urine fell precipitately (values of 1.002–1.004 were common) after the Eck fistula operation indicates that the greater water exchange does not result from the liberation and excretion of stored salts. Further, the serum osmotic pressure of our animals, as determined by the freezing point method, remained within normal limits.

It is well known that there is a considerable variation in Eck fistula dogs, some showing little observable change, others exhibiting symptoms promptly and dying within a few months, while the majority run an intermediate course. Therefore it is not surprising that the effect on water exchange was also variable. We noted that the animals which drank and excreted the largest amounts of water post-operatively exhibited the characteristic symptoms, while those in which the water exchange was little affected showed relatively little change from the normal in other respects.

The duration of the effect on water exchange was not carefully studied. In dog E-1 the intake and output were still approximately double the control level in the ninth post-operative month, although greatly decreased from the 800 per cent rise that occurred immediately after the operation. Dog E-7 was used primarily for other studies, but 10 months after operation it was noted that his voluntary intake per kilo was approximately 3 times the average of our normal series and more than twice as great as the maximum normal.

The observed correlation between plasma chloride dilution after water by mouth and voluntary 24 hour intake was to be expected if the increased water exchange is secondary to a reduction in the ability of the liver to store water. Baldes and Smirk (9) have presented evidence indicating that urine formation is largely controlled by changes in the total osmotic pressure of the blood. The greater blood dilution and more prompt and complete urinary excretion of ingested water in animals with liver damage supports this concept. It also seems that interference with the water storage function of the liver, resulting in a more prompt elimination of ingested water, leads to an earlier reappearance of thirst. Repetition of the cycle brings about an increase in voluntary water consumption and urinary ouput over long periods of time.

SUMMARY

Establishment of Eck fistulae in dogs leads, in the majority of instances, to a lasting increase in voluntary water intake and urinary output. Chloroform poisoning acts similarly, while a dummy Eck fistula operation, splanchnic section, or bile duct ligation is without effect. The increased water exchange is correlated with a greater dilution of the plasma chlorides after oral administration of water. The effect appears to be secondary to interference with the water storage function of the liver, with a consequent prompt elimination of water and reappearance of thirst.

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THE GLUCOSE UTILIZATION OF PHLORIDZINISED DOGS AFTER HEPATECTOMY

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The rate of glucose utilization by the tissues of the phloridzinised dog has an important bearing on the theory of the intermediary metabolism of foodstuffs. After three days of fasting and phloridzin administration the readily-available glycogen of the body has been removed, and glucose that is excreted after this comes from non-carbohydrate sources. From the nitrogen excreted in the urine, the amount of protein catabolised may be determined, and if it be assumed that no sugar can arise from fat the urinary glucose must come from protein catabolised. The dextrose: nitrogen ratio of the urine of such animals is 3.65:1 and since each gram of urinary nitrogen is supposed to come from 6.25 grams of protein, it is claimed that 6.25 grams of protein can give rise to 3.65 grams sugar. However in such calculations no consideration is given to the glucose which may be oxidized in the tissues, presumably it being assumed that none has such a fate. Wierzuchowski (1927) and Deuel, Wilson, and Milhorat (1927) have shown that the phloridzinised dog is capable of oxidizing administered glucose. The question to be answered is, are the tissues of the phlorizinised animal actually utilizing glucose when none has been administered, and the only source of it is that formed in the body from other foodstuffs. All evidence today points to the liver as the site of this transformation. The amount of such new formed sugar will equal the sugar excreted plus the glucose oxidised by the tissues. If the liver be removed the source of glucose within the body is eliminated and in order to maintain the blood sugar level constant, glucose must be supplied to the animal at a rate at least equal to the rate of excretion by the kidneys. If more than this must be given to maintain the level then we may presume that the tissues are utilizing the substance.

METHOD. Stated simply the method consists of producing the phloridzinised state (three or more days of fasting and phloridzin), next removing the liver and then maintaining, with intravenous glucose, the blood sugar level that was found immediately before operation. In some experiments the kidneys were removed and in the others the bladder was emptied and

washed after operation and urine collected thereafter, to determine the sugar excreted. In the later experiments blood lactic acid and muscle glycogen determinations were made on samples taken at the beginning and end of the experimental period, in order to check up on every possible source or loss of carbohydrate.

Phloridzin was administered daily, subcutaneously, the dosage being one gram suspended in olive oil. Hepatectomy was performed by the method of Markowitz and Soskin (1927) as modified by Drury (1929). Blood sugar determinations were carried out by the method of Folin and Wu. For glucose estimation on the urine the Shaffer-Hartmann method was used. For muscle glycogen determinations whole muscles were used, the flexor carpi radialis of the fore leg is suitable for this purpose: it weighs 2 to 3 grams, and can be removed, using local anesthesia, by cutting its tendons at either end. The muscle of one leg is taken at the beginning of the period and this is compared with the corresponding muscle of the other leg which is taken at the end. Pflüger's procedure was followed, with Shaffer-Hartmann determination of glucose after hydrolysis. Lactic acid determinations on blood and urine were carried out by the method of Friedman, Cotonio and Shaffer as modified by Wendell (1933).

The actual routine of the experiments was as follows: The dog, after the phloridzin period, was brought to the laboratory and a blood sample was taken for sugar estimation. Hepatectomy was then done under ether anesthesia. As soon as the liver vessels were tied, constant intravenous injection of a 5 or 10 per cent glucose solution was started, this being delivered from a burette, through a dropping funnel, to a needle kept in place in one of the hind leg veins. Blood sugar estimations were done every half hour or less, and the injection rate was adjusted accordingly, the attempt being made to keep the blood sugar level the same as that found immediately before the operation. As soon as the operation was completed, the urinary bladder was washed out, a blood sample was taken for lactic acid, and a muscle for glycogen, determinations. The animal was then carried for several hours, and the experiment was completed by collecting all the urine secreted since the first washing, and by taking samples for determination of muscle glycogen and blood lactic acid.

RESULTS. The following is the protocol of a typical experiment in which the kidneys were not removed.

Dog 1. 4–15–36 to 4–19–36. Fasted and given 1 gram phloridzin in olive oil subcutaneously every day (5 days).

4–20–36	9:00 a.m.	Weight 12.7 kgm. Blood sugar 50.
	9:05	Ether started for hepatectomy.
	9:55	Portal vessels tied. Intravenous injection of 10 per cent glucose started, burette reading at 0.0.
	10:12	Blood sugar 72. Burette reading 3.4.

	10:53	Blood sugar 78. Burette reading 10.1.
	10:55	Operation ended.
	11:20	Muscle taken from right fore leg. Glycogen concentration = 242 mgm. per cent.
		Blood lactic acid = 121 mgm. per cent.
	11:27	Blood sugar 56. Burette reading 13.0.
	11:44	Bladder washed and urine collection started. Burette reading 14.5.
	12:10 p.m.	Blood sugar 57. Burette reading 16.9.
	12:44	Blood sugar 52. Burette reading 20.3.
	1:12	Blood sugar 47. Burette reading 22.8.
	1:43	Blood sugar 42. Burette reading 26.4.
	2:15	Blood sugar 45. Burette reading 31.6.
	2:45	Blood sugar 57. Burette reading 37.8.
	3:14	Blood sugar 60. Burette reading 42.5.
	3:53	Blood sugar 51. Burette reading 47.0.
	3:50	Blood lactic acid = 216 mgm. per cent.
	3:57	Urine collection stopped. Bladder washed.
	4:20	Muscle removed from left fore leg. Muscle glycogen = 189 mgm. per cent.

The urine passed between 11:44 a.m. and 3:57 p.m. had a volume of 60 cc. and contained 1,488 mgm. glucose and 258 mgm. lactic acid. During this period 3,250 mgm. were injected. The difference 1,762 mgm. represents glucose used by the animal. (The drop in blood sugar was slight so that the correction for this would be small.)

The following is the protocol of an experiment in which the kidneys were removed at the same time as the liver.

Dog 2. 12-16-35 to 12-19-35. Phloridzin and fasting as in previous experiment (4 days).

12-20-35	9:20 a.m.	Weight 6.35 kilos. Blood sugar 46 mgm. per cent.
	9:25	Ether. Operation—hepactectomy and double nephrectomy.
	9:53	Portal vessels tied and constant intravenous injection of 5 per cent glucose started. Burette reading 0.0.
	10:11	Blood sugar 56. Burette reading 4.0.
	10:35	Operation finished.
	10:43	Blood sugar 59. Burette reading 7.2.
	11:11	Muscle taken glycogen concentration = 187 mgm. per cent.
	11:15	Blood sugar 55. Burette reading 10.2.
	11:18	Blood lactic acid = 149 mgm. per cent.
	11:45	Blood sugar 51. Burette reading 12.8.
	12:15	Blood sugar 43. Burette reading 14.0.
	12:45	Blood sugar 42. Burette reading 18.0.
	1:15	Blood sugar 44. Burette reading 22.0.
	1:45	Blood sugar 41. Burette reading 27.2.
	2:17	Blood sugar 45. Burette reading 35.0.
	2:52	Blood sugar 38. Burette reading 38.0.
	2:50	Blood lactic acid. 189 mgm. per cent.
	2:52	Muscle taken glycogen concentration = 90 mgm. per cent.

Between 11:15 a.m. and 2 : 52 p.m. the animal received 1,410 mgm. glucose. The

blood sugar level dropped during this period so that the utilization during this period was somewhat higher than this.

The results of the other experiments will be given briefly.

Dog 3. Three days phloridzin and fasting.
Hepatectomy only. Weight at operation 17 kilos.
In 4 hours and 28 minutes received 8,950 mgm. glucose intravenously.
In this period excreted 4,750 mgm. glucose. Blood sugar before operation 60. At beginning of experimental period 63; at end, 54. Muscle glycogens and blood lactic acids not done.

Dog 4. Three days phloridzin and fasting.
Hepatectomy and nephrectomy. Weight at operation 5.9 kilos.
In 5 hours and 58 minutes received 5,138 mgm. glucose intravenously. Blood sugar before operation 46. At beginning of experimental period, 31; at end, 38.
Muscle glycogen at beginning 595 mgm. per cent; at end, 362 mgm. per cent. Blood lactic acids not done.

Dog 5. Five days of phloridzin and fasting.
Hepatectomy only. Weight at operation 11.8 kilos.
In 3 hours and 20 minutes received 4,350 grams glucose intravenously.
During this time excreted 1,000 mgm. glucose.
Used 3,350 glucose.
Blood sugar before operation 54. At beginning of experimental period, 51; at end, 68.
Muscle glycogen at beginning 219 mgm. per cent; at end, 132 mgm. per cent.
Blood lactic acid at beginning 65 mgm. per cent; at end, 63 mgm. per cent.
Urine contains 10 mgm. lactic acid.

Discussion. The results of these experiments suggest that the tissues of the phloridzinised dog utilize glucose even when the blood sugar is kept low. Can we say that it is oxidized? It has not gone into extra muscle glycogen since the value regularly dropped. The blood lactic acid (and with it the tissue lactic acid) usually rose, but on the average, the amount of lactic acid that this corresponds to, together with that excreted in the urine, is about equivalent to the loss in muscle glycogen.

The average glucose utilization for all our animals amounted to 75 mgm. per kilo per hour or 1.8 gram per kilo per day. This represents something like 10 per cent of the metabolic needs of the animals. It is of interest to calculate the D:N ratios of our dogs when this amount of glucose is added to that which is excreted. Our dogs when completely phloridzinised (before the operation) had an average D:N ratio of 3.89 and excreted 3.07 grams glucose, and 0.808 gram nitrogen, per kilo per day. If we add the amount of glucose used by the tissues as calculated above (1.8 gram) to that excreted, we obtain the sum of 4.87 grams which when divided by 0.808 gram gives a D:N ratio of 6.04. If the conversion of fatty acids to glucose be denied, this would require an almost complete conversion of protein to

glucose (in contrast to the conventional 58 per cent). Some of this extra glucose may come from the glycerol fraction of the fats, though at most probably not more than one-third of this extra glucose could arise from this source.

On the other hand our results give no support to the concept that practically all foodstuffs, including fatty acids, are converted to glucose by the liver before utilization by the tissues. The glucose which we had to supply after hepatectomy represents a small fraction of the metabolic needs of the animal. The work of Bollmann, Mann, and Magath (1926) indicates that little if any of the energy turnover of liverless animals comes from protein. We are forced to the conclusion that most of the energy supply in our animals was from the direct utilization of fats.

SUMMARY

Completely phloridzinised dogs (3 to 5 days fasting and phloridzin) after hepatectomy require the administration of glucose to maintain the blood sugar level existing just before operation. The amount required averages 75 mgm. per kilo per hour. Analyses for lactic acid and muscle glycogen do not account for this glucose requirement.

If this glucose utilization by the tissues of the phloridzinised dog be added to that excreted, we obtain D:N ratios of the order of 6.

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OBSERVATIONS ON THE BLOOD FLOW AND GASEOUS METABOLISM OF THE LIVER OF UNANESTHETIZED DOGS[1]

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The flow of blood through the liver has been determined by a number of observers who have worked on anesthetized animals. Particularly pertinent among these studies are those of Schmid (1908), Burton-Opitz (1910, 1911), Barcroft and Shore (1912), Grab, Janssen and Rein (1929) and Schwiegk (1932). Schmid determined the flow of blood through the portal vein of deeply anesthetized dogs and cats by the use of a stromuhr. The measurements by Burton-Opitz were obtained after connecting a recording stromuhr with the hepatic artery in some experiments and with the portal vein in others. In these experiments, on anesthetized dogs, the portal vein was occluded for approximately ten minutes while connections were made to the stromuhr. Barcroft and Shore used anesthetized cats in their studies. The inferior vena cava was occluded between the adrenal veins and the hepatic veins and the flow was diverted into the jugular or brachial vein. Cannulae were then placed in the inferior vena cava above the point of occlusion and in a side branch of the portal vein and the flows through these two vessels were determined following temporary constriction. From these measurements, together with oxygen analyses, the gaseous metabolism was calculated. The average arterial blood pressure at the time of the measurement of the hepatic blood flow in Barcroft and Shore's experiments was 78 mm. mercury. Grab, Janssen and Rein working with anesthetized dogs used the thermoelectric stromuhr. No blood samples were taken for gas analyses. Schwiegk also employed the thermoelectric stromuhr on anesthetized dogs in determining the hepatic blood flow.

The purpose of the present experiments was to determine the blood flow and gaseous metabolism of the liver of unanesthetized dogs. A modification of a method previously described by Mason, Blalock and Harrison (1936) for measuring the renal blood flow plus the use of an instrument described by Horine (1928) made the study possible.

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METHOD. Dogs were used as the experimental animals in all instances. They were trained to lie quietly upon the table during the observation period. No food had been allowed for 18 hours preceding the experiment. No anesthetic was used except novocain locally at the sites of incisions in the neck. Aseptic technique was employed in the experiments in which additional studies were to be performed on subsequent dates. The animals gave no evidence of pain during the experiments and were observed to eat, play or fight following the completion of the studies.

The principle of the method consisted of producing a temporary blockage of the inferior vena cava both above and below the entrances of the hepatic veins and diverting the blood during this brief period into a cannula which had been inserted through the right external jugular vein. The cannula was made of brass and was 45 cm. in length. The distal end (liver) was closed and the proximal end (neck) was left patent. Slightly proximal to the distal end the cannula was equipped with two externally expanding balloons separated by a distance of 7.5 cm. These balloons (made from Penrose tubing) could be inflated by means of small internal tubes passing to the proximal end of the cannula. Between the balloons the cannula was perforated by fifteen large openings, leaving only enough cannula wall to render it rigid. The cannula had a capacity of approximately 1000 cc. of blood per minute at a level of minus 30 cm. At a preliminary operation, a number of days previously, an instrument described by Horine and designed to permit withdrawal of blood from the portal vein without surgical exposure was implanted. This device may be left in place for an indefinite period and apparently affords no discomfort to the animal nor does it interfere with the portal circulation. At the same time an untied ligature was placed around the hepatic arteries. The two ends of the ligature were brought out through the anterior abdominal wall.

At the beginning of the experiment, with the dog lying on his back, novocain was introduced over the sites of the two external jugular veins. Each vein was exposed and a short cannula, directed towards the heart, was introduced on the left. This was followed by the intravenous injection of 200 mgm. of heparin (Connaught Laboratory). The cannula equipped with the balloons was then introduced into the right external jugular vein and was passed through the superior into the inferior vena cava until its tip approximated the entrances of the renal veins. The proximal end of the cannula was closed during this procedure which was followed by aspiration of the air within the lumen of the tube.

With the aid of a fluoroscope, the cannula was then withdrawn until the opening into the tube closest to the uppermost balloon could just be seen above the diaphragm. From previous measurements, this signified that the uppermost balloon was between the entrances of the hepatic veins and the heart and the distal balloon was between the adrenal and hepatic

veins. This being true, the only blood which could enter the cannula when the balloons were inflated was that which came from the hepatic veins plus a small amount which came through the veins of the diaphragm. The cannulae in the jugular veins were connected by a T-tube with a side-arm of rubber tubing. This permitted through and through circulation.

After the cannula had been placed in correct position and the proper connections had been made, the rate of flow was determined. The lower balloon was inflated, the rubber tubing connecting the through and through circulation was occluded and simultaneously the blood was allowed to flow from the rubber tube side-arm into a calibrated vessel. This was followed immediately by the expansion of the upper balloon. After approximately 40 cc. of blood had escaped, the time required for the passage of 50 cc. of blood was determined. The observation was repeated a number of times with the outflow tube at different levels. The usual level was 30 cm. below the left auricle; however, this was governed by the capacity flow which was determined with the balloons uninflated. A position was chosen at which the observed flow was less than the capacity flow, but at which the pressure in the outflow system was not made sufficiently negative to cause marked pulsations due to collapse of the vena cava and hepatic veins. After satisfactory checks had been obtained, samples of blood for gas analyses were obtained. The hepatic vein sample was obtained from the tube through which the flow had been measured. Arterial blood was taken from the femoral artery and portal vein blood was drawn by introducing a needle through the instrument overlying the portal vein. All samples were taken with anaerobic technique. Approximately 200 cc. of blood were obtained from a donor prior to the experiment in order to have a store for replacement at the time of the measurements of the flow, and after sampling. It was injected into the left jugular vein at the time that the flow was being measured and at approximately the same rate. This kept the blood volume of the animal from being significantly altered at any time during the experiment. The oxygen contents, oxygen capacities and CO_2 contents of the blood from the three sources were determined.

Following the sampling procedure further measurements of the blood flow were made. The hepatic arteries were then occluded by pulling the ends of the ligature coming out of the abdominal wall, and 30 seconds later the flow was again measured. The difference in flow before and after occlusion was taken to represent that portion of the flow contributed by the hepatic artery.

In calculating the oxygen consumption of the liver the oxygen content of the hepatic vein blood was corrected on the basis of the oxygen capacity difference between it and the arterial and portal vein blood according to the method employed by Van Slyke et al. (1934). Eventually most of the dogs were sacrificed in order to obtain the weights of the livers. In some

TABLE 1

Hepatic blood flows and oxygen consumptions of dogs in which the hepatic arterial contribution to total flow was determined

DOG	WEIGHT	CORRECTED OXYGEN CONTENTS			A-V (O_2)		CARBON DIOXIDE CONTENTS			APPARENT RESPIRATORY QUOTIENT
		Arterial	Portal vein	Hepatic vein*	Arterial hepatic vein	Portal vein; hepatic vein	Arterial	Portal vein	Hepatic vein	
	kgm.	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	
36	13.71	16.42	11.76	8.52 8.16	7.90	3.60				
26	18.79	20.57	16.04	11.47 11.38	9.10	4.66	39.80	43.15	45.90	0.61
26	18.50	15.57	11.20	6.45	9.12	4.75	41.68	45.75	49.14	0.74
17	16.10	18.00	12.43	5.04 5.02	12.96	7.41	36.20	42.82	46.44	0.52
17	15.82	13.93	6.28	4.65 4.58	9.28	1.70	31.14	36.82	40.18	1.36
195	13.20	13.28	6.47	4.12 4.29	9.16	2.18	39.98	43.68	45.50	0.70
27	14.28	15.55	9.38	5.03 4.79	10.52	4.59	37.04	42.62	44.56	0.53

DOG	WEIGHT, LIVER	HEPATIC BLOOD FLOW					PER CENT TOTAL FLOW FROM HEPATIC ARTERY	HEPATIC OXYGEN CONSUMPTION		
		Total	Hepatic artery	Portal vein	Per kgm. dog	Per kgm. liver		Per dog	Per kgm. dog	Per gram liver
	grams	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*		*cc. per minute*	*cc. per minute*	*cc. per minute*
36	553	476	84	392	34.7	0.86	17.6	20.75	1.51	0.038
26	546	612	77	535	32.6	1.12	12.6	31.94	1.70	0.058
26	546	462	70	392	25.0	0.85	15.2	25.00	1.35	0.046
17	535	313	48	265	19.4	0.58	15.4	25.85	1.61	0.048
17	535	385	88	297	24.3	0.72	22.9	13.22	0.84	0.025
195	482	487	119	368	36.8	1.01	24.5	18.92	1.43	0.039
27		469	99	370	32.8		21.1	27.40	1.92	

* The observed hepatic venous content is corrected in one case for the oxygen capacity difference between arterial and hepatic vein blood, and in the other for the difference between the portal and hepatic vein blood. Thus to calculate the A-V (O_2) there are two values for hepatic venous oxygen content.

instances this immediately followed the experiment and the position of the cannula was checked at autopsy.

RESULTS. The complete observations on total flow, flow following constriction of the hepatic artery, and gaseous metabolism were made on five dogs. In another series of animals similar observations were made except that the occlusion of the hepatic artery was omitted. In a further series the total flow and the flow after hepatic arterial constriction were

TABLE 2

Hepatic blood flows and oxygen consumptions of dogs in which the hepatic arterial contribution to total flow was not determined

DOG	WEIGHT	LIVER WEIGHT	CORRECTED OXYGEN CONTENTS			A-V (O_2)		HEPATIC BLOOD FLOW			HEPATIC OXYGEN CONSUMPTION*		
			Arterial	Portal vein	Hepatic vein†	Arterial hepatic vein	Portal vein: hepatic vein	Total	Per kgm. dog	Per gram liver	Per dog	Per kgm. dog	Per gram liver
	kgm.	*grams*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*vols. per cent*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per minute*	*cc. per min. ute*	*cc. per minute*
					12.10								
17b	14.20		20.04	16.59	12.04	7.94	4.55	438	30.8		22.91	1.61	
					6.88								
55	14.20	579	20.52	15.20	6.72	13.64	8.48	266	18.7	0.46	25.28	1.78	0.044
					5.04								
56	15.56		18.86	13.65	5.17	13.82	8.48	342	22.0		32.63	2.10	
					11.40								
6	11.50		18.17	16.73	11.77	6.77	4.96	344	29.9		18.31	1.59	
					2.62								
4	12.25	433	15.05	7.06	2.63	12.43	4.43	319	26.0	0.74	19.25	1.57	0.044
					5.41								
4	12.31	433	13.64	10.61	5.35	8.23	5.26	484	39.3	1.12	28.35	2.30	0.065

* Calculated on the basis of the hepatic arterial flow amounting to 20 per cent of the total flow. This is the average of 10 observations.

† See footnote table 1.

determined, but no blood samples were taken. Finally a number of animals were used for the purpose of determining only the total flow.

The results of the observations on those dogs in which the complete hepatic blood flows as well as the gaseous metabolisms were determined are given in table 1. In table 2 the results are given for the series in which the occlusion of the hepatic artery was omitted. The oxygen consumptions in these instances were calculated on the basis of the hepatic arterial

contribution to total flow being the average of all experiments in which this was determined, some of which are given in table 1.

Discussion. *Total liver blood flow.* The total hepatic vein outflow was determined in 24 instances. The weights of the dogs varied from 11.5 to 18.8 kgm. and the liver flow varied from 216 to 612 cc., the average being 387 cc. The flow per kilogram of body weight varied from 17 to 39.3 cc., the average being 27.0 cc. In the instances in which the weight of the liver was determined, the flow per gram of liver tissue per minute varied from 0.46 to 1.12 cc., the average being 0.82 cc. Eight of the 15 values fell between 0.72 and 0.86 cc.

These rates of flood flow are much greater than those of Barcroft and Shore and are of the order of those obtained by Burton-Opitz, by Grab, Janssen and Rein and by Schwiegk.

Hepatic arterial blood flow. The hepatic arteries were constricted in ten instances, repeat determinations being performed on two dogs at intervals of several days. The reduction in the hepatic vein flow which resulted from this procedure varied from 12.6 to 24.5 per cent of the total, the average being 19.5 per cent. Conversely, the flow of blood through the portal vein constituted 80.5 per cent of the total liver flow.

In fasting animals, Barcroft and Shore found that the hepatic arterial flow constituted 34 per cent of the total flow through the liver. Burton-Opitz did not determine the portal vein flow and the flow through the hepatic artery in the same animal but concluded from a number of studies on each that 30 per cent of the total quantity of blood passing through the liver came from the hepatic artery. Grab, Janssen and Rein found that the average contribution of the hepatic artery was 19.0 per cent of the total liver flow, which is in close agreement with our findings. The variations we have observed in this function are also of the order observed by those authors and by Schwiegk.

Constriction of the hepatic arteries resulted in a marked diminution in the oxygen content of the blood in the hepatic veins. In several instances the values were less than one volume per cent during the occlusion period.

As has been stated, the portal flow through the liver was measured 30 seconds following the occlusion of the hepatic arteries. Measurements made several minutes later with the constriction still maintained usually showed that the flow had returned to the pre-occlusion rate.

Oxygen consumption of the liver. The total oxygen consumption of the liver varied from 13.2 to 32.6 cc. per minute, the average of all experiments being 23.8 cc. per minute. The oxygen consumption per gram of liver per minute varied from 0.025 to 0.065 cc., the average being 0.045 cc.

Barcroft and Shore found that the oxygen consumption of the livers of anesthetized cats which were fed 18 hours before the experiment varied

from 0.024 to 0.050 cc. per gram per minute and that the hepatic artery was the dominating source of oxygen supply to the liver.

Schwiegk, in experiments on dogs anesthetized with chloralose, obtained portal vein blood oxygen unsaturations of about 30 per cent and hepatic vein unsaturations of about 50 per cent, and concluded that on the average the hepatic artery supplied about 40 per cent of the oxygen utilized by the liver. In the series of experiments for which the data are given in table 1, the hepatic artery supplied between 22 per cent and 38 per cent of the oxygen in five instances and 58 per cent and 62 per cent in two others. All these dogs were presumably in the fasting state.

Respiratory quotient. In those cases where the hepatic arterial contribution to the total flow was actually determined (table 1) the respiratory quotients were computed. The values obtained were variable, and it is doubtful if any interpretation can be made of them; however, in some preliminary experiments constant gross changes were noted after administration of food, and this point is being further investigated.

The method which has been described has certain advantages as a means of studying hepatic physiology. It affords the opportunity of obtaining relatively large samples of hepatic vein, portal vein and arterial blood simultaneously from the unanesthetized animal and at the same time permits the determination of the portal and arterial supply in relation to the total flow. It has not been satisfactory as a means of studying the transient or rhythmic changes in the blood supply of the liver for which purpose the thermo-electric stromuhr is ideally suited.

SUMMARY

The hepatic oxygen consumption and the total, portal, and arterial blood flow through the liver of the unanesthetized dog have been determined.

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THE DISTRIBUTION OF GLUCOSE IN BLOOD

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Olmsted (1) has reported that when proper precautions are taken the blood cells of various species, including man, contain little or no glucose. All of the glucose is in the plasma. He (2) has further stated that the blood cells of various species differ in their permeability to glucose after the addition of oxalate to the blood. In this respect the corpuscles of man are least resistant to and more readily disturbed by the addition of this anticoagulant. This was demonstrated by a study of sugar distribution in oxalated blood at various time intervals.

These findings of Olmsted are at variance with those generally accepted regarding blood sugar distribution. They are of importance in connection with many physiological investigations, for example, those on kidney secretion.

A reinvestigation of the above work (2) using blood as ordinarily drawn with oxalate as the anticoagulant, failed to confirm Olmsted's findings that human blood cells readily become permeable to glucose, in contrast to rabbit blood cells, which are reported to do so slowly. In twelve experiments using human blood, in which the sugar distribution was studied up to three hours, and in three experiments with rabbit blood, in which the sugar distribution was studied up to one and one-half hours, there was no evidence of an increased penetration of sugar into the cells. However, in five experiments with pig blood, in which the sugar distribution was studied up to eight hours, the findings of Olmsted were confirmed.

Our next experiments were carried out using the special precautions outlined by Olmsted. Here again, in the case of human and rabbit blood, his findings (1, 2) were not confirmed. Our results are given in table 1. It should be pointed out that the determination of whole blood sugar was done only on the oxalated material.

The methods used for the determination of glucose were those of Benedict (3, 4). These methods are as specific for glucose as those used by Olmsted. A problem of this nature fundamentally resolves itself down to a determination of differences in sugar content of oxalated and unoxalated (no anticoagulant) plasmas. No difference in glucose content in such plasmas was found in this work (table 1).

TABLE 1

Milligrams of glucose in plasma and corpuscles given as in 100 cc. of whole blood

HEMATOCRIT	WITHOUT ANTICOAGULANT		OXALATED		AFTER OXALATE*	
	Plasma	Corpuscles	Plasma	Corpuscles	Plasma	Corpuscles
			Human			
per cent						
42	55	32	55	32		
48	48	39	48	39		
44	51	34	52	33		
50	33	29	32	30		
42	52	30	51	31		
49	41	33	40	34		
50	44	36	43	37		
46	50	36	50	36		
45	44	31	45	30	41	28
47	41	31	41	31	38	29
42	50	30	50	30		
41	47	27	47	27	44	24
46	50	36	51	35	48	34
47	48	37	48	37	46	35
46	49	33	49	33	45	32
45	57	38	57	38	53	36
43	60	39	60	39	57	36
46	44	32	44	32	41	30
			Rabbit			
38	104	5	102	7		
35	91	13	90	14		
35	120	17	119	18		
37	52	12	51	13	44	8
37	88	17	87	18	76	13
30	50	9	49	10		
36	116	19	115	20	103	15
31	117	11	118	10	102	8
31	97	14	97	14	90	11
34	104	11	103	12		
31	110	14	109	15	96	10
37	147	20	147	20	139	16

* For human bloods, 30 minutes; for rabbit bloods, 75 minutes.

CONCLUSION

The findings of Olmsted that human and rabbit blood corpuscles contain no glucose are not confirmed. Nor are his findings that oxalate changes the permeability of such blood cells to glucose verified.

For drawing the samples of human blood, I am indebted to Doctors Brown and Lane; for the rabbit blood, to Mr. J. F. Reinhard. I am also grateful to those students from whom blood was obtained.

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A COMPARISON OF THE ELECTROGRAM OF THE OPTIC CORTEX WITH THAT OF THE RETINA[1]

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Certain visual phenomena can be recorded not only from the eyeball itself in the usual electro-retinogram, but from other stations in the optic pathway such as the optic nerve (Adrian and Matthews, 1927) or single active fibres before they leave the eye as part of the optic nerve (Hartline, 1935). Further, it is possible to determine some aspects of retinal behavior by recording from the striate area of the cerebral cortex, for most of the latency of the cortical response represents time consumed in the eye (Bartley 1934). Stimulus conditions which vary the latency of retinal response effect quite similar changes in the cortical response. Certain comparative details of the two responses are presented to show the possibility of using the first wave in the cortical response for measuring activity as far back as the eye.

The typical retinal response to a long flash of light is as in figure 1A. To the beginning of the flash a wave complex occurs called the *on* response, and to the cessation another called the *off* response. The *on* is composed of the *a* and *b* waves, the *a* not appearing in the records of all animals or of all preparations. If the stimulus is long, a subsequent gradual development of potential may occur (the *c* wave), and if the flash is weak or short no *off* response (*d* wave) appears. Several experimental procedures, such as asphyxiation, etherization, temperature manipulation, and massage of the eyeball markedly alter the shape of the whole retinal record, a result which has led to the analysis of the picture into three components, PI, PII and PIII (Granit, 1933, modified after Piper, 1911). Our interest is in the *b* wave of the *on* effect, which Granit believed to be produced by PII, the only one of the three processes concerned with the discharge of impulses into the optic nerve; and also in the possible *off* effect. It is the *b* wave of the retinal record that compares most closely with the cortical response, but since the cortical record exhibits an *off* effect, its precursor might be expected in retinal neural action. Granit, however, attributes

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the retinal *off* effect to a rapid return of the negative PIII wave to the base line and has excluded PIII from connection with neural discharge, except as an inhibitor of PII. From this, no cortical *off* effect might have been expected, but on the contrary it is far more pronounced than the retinal *off* response, which findings suggest a reinterpretation of some of the findings of the electro-retinogram technique. The finding of a cortical *off* response is in keeping with those of Hartline mentioned below, in which single afferent fibres which are to compose the optic nerve exhibit an *off* response.

It will be noted that all of the transitions in the retinal record are slow, so the components of the *b* wave are long. It is possible that the record includes not only neural potentials but other processes, though it does not

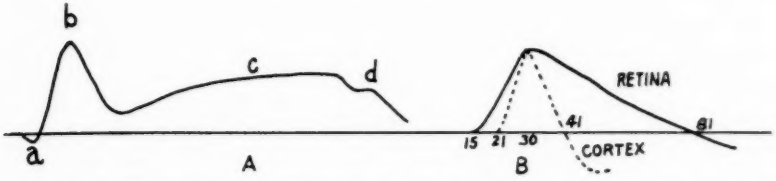

Fig. 1. A. Shows a typical response to a flash of light a few seconds long recorded from the eyeball. In some animals a small negative deflection, the *a* wave, appears first. The *b* wave occurs in all, and under appropriate conditions the slow *c* wave. If the light lasts long enough and is bright enough a further small deflection in the general course of the potential appears (the *d* wave) at the cessation of the light. In all cases the potentials are slow and do not reveal the complicated events which we know are occurring in the eye.

B. A comparison of the time relations of the *b* wave of the retinal record and the first potential of the cortical response. The onset of the *b* wave is often very gradual but definitely precedes the onset of the cortical wave. Their peaks may coincide, however. Some difficulty is experienced in determining the exact peak of the *b* wave, for it is somewhat broad. Here again the relative abruptness of the cortical response is demonstrated.

seem to be readily analyzed in ways essentially different from the method already described.

The optic nerve record, like the retinal, portrays the gross activity of the nerve, and for analysis, ways must be sought to divide the nerve into its anatomical constituents, the fibres. This in effect has been done in Hartline's experiments in recording from afferent fibres just before their collection into the optic nerve. By this technique he has been able both to record discrete discharges in single fibres and to study the activity of local retinal areas. The repetitive end organ discharge which is recorded in the optic nerve is rearranged as it reaches the first way station, the lateral geniculate body. Facilitation in this nucleus condenses the afferent discharge which arrives at the onset of activity (Bishop, 1933; Bartley,

1935) so that the cortical response is a more abrupt and shorter wave than the retinal *b* wave, a result working to advantage in many experiments.

METHOD. All of the records were obtained by the use of the cathode-ray oscillograph and vacuum tube amplifier on rabbits held in light ether anesthesia by tracheal cannulae.

The flashes of light were regulated by a sector disc whose speed could be varied and measured, the latter by a Weston tachometer. The beam of light emerged from a narrow slit, so that the onset of the flash occupied only 1.5–8 sigmas depending on the speed of the disc. The light illuminated the face of a biconvex lens whose face subtended a visual angle of 11°20′. The visual angle concerned us only in the production of a large flux of light and not in any direct spatial way, for it has been shown that both retinal and cortical records are the result, for the most part, of the activity of the stray light illuminating the retina in general (Bartley, 1935; Fry and Bartley, 1935). The maximum brightness was 30,500 c/ft^2 and unless otherwise specified was used in all experiments except the comparisons between the cortical *off* and *on* responses. The dim flashes were 0.004 of the maximum. In the comparisons between cortical *off* and *on* responses the visual angle was 7° and the brightness was 1280 c/ft^2. The pupillary aperture in each case was in the neighborhood of 15 sq. mm. Some light also fell on the cornea outside the pupil.

EXPERIMENTAL. The following experiments give a comparison of the gross features of the retinal *b* wave (hereinafter called the retinal record or retinal *on* effect) and the cortical record and show how the two responses behave under the same manipulation of stimulus conditions.

1. *Comparison of gross time relations.* If a 115$^\sigma$ flash once every 1800$^\sigma$ is presented, the *peaks* of the retinal and cortical *on* effects coincide in time. In one animal, for instance, they were both at about 30$^\sigma$, the retinal effect of course *beginning* sooner and *ending* much later than the cortical. The starts of the two waves were 15$^\sigma$ and 21$^\sigma$ respectively for retina and cortex, and the completions of their positive phases 81$^\sigma$ and 41$^\sigma$ (fig. 1B). Inasmuch as both effects are diphasic, these last values do not mark the completion of the whole effect. Flashes which approach the critical duration shorten the responses a little. And, too, the onset of the flash may be made gradual enough to practically obliterate the *on* response as a definite wave in the record. The difference in results with short and long stimuli will be brought out more definitely in the next experiments to be described.

2. *Reduction of amplitude and increase in latency.* Among the factors that will decrease the amplitude and increase the latency of response is the repetition of the flash at short intervals. This is done by simply increasing the speed of the sector disc. Figure 2 shows the results of using various intervals between the origins of the flashes, ranging from 1800$^\sigma$

to 45$^{\sigma}$. Part of this range was covered both by flashes equal in length to the interval between them and by short flashes from 11 to 4$^{\sigma}$, a light-dark ratio of 0.006 as compared to 0.5000. The curves are from one set of experiments on a single animal; though the trend of the results is typical, the absolute values cannot be taken as more than representative. The points to be gained from the curves are that shortening the interval be-

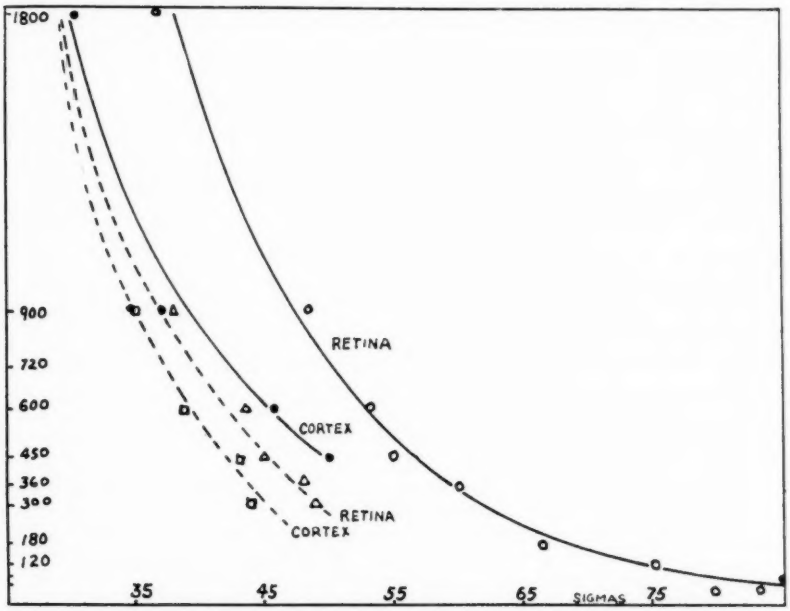

Fig. 2. The relation between flash intervals and the latency of the peaks of the retinal and the cortical records. The ordinate represents the flash intervals and the abscissa the latency, known as the "implicit time" in connection with cortical records when so measured. The solid lines are for cases of dark and light intervals of equal length. The broken lines are for short flashes (11-4$^{\sigma}$) in which the light-dark ratio is 0.006 rather than the former 0.500. It will be noted that with short flashes the retinal and cortical peaks more nearly coincide. It is sufficient to see that the curves do not diverge greatly under similarly changing conditions as through some possible function of the geniculate station.

tween flashes increases the latency for both the retinal and cortical responses as measured to the peak of the waves. The peak of the retinal response is temporally shifted more than the cortical peak, though the two peaks often arise at about the same time with short flashes.

As the interval between the beginnings of the flashes is reduced from 1800$^{\sigma}$, the amplitude of both retinal and cortical response dwindles.

Figure 3 shows the manner in which the reduction occurs. Curve *a* represents the positive phase of the retinal response, and *b* both phases. Curve *c* represents the positive phase in another animal, using shorter intervals, and *d* the dwindling of the cortical response in the same animal. The curves *a* and *b* are measured from photographs, and *c* and *d* upon the face of the oscillograph. The curves indicate that the retinal response disappears with an interval between 45 and 30$^{\sigma}$, which in terms of flicker would represent a critical frequency of about 22 to 33.

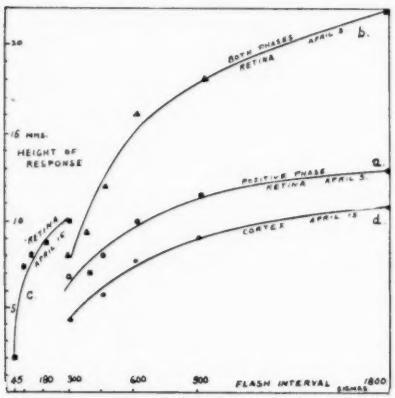

Fig. 3

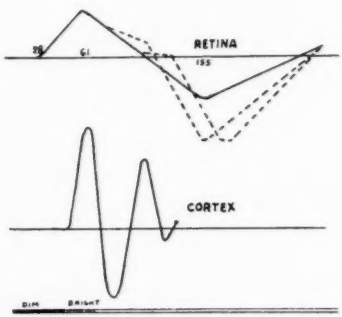

Fig. 4

Fig. 3. The relation of amplitude to flash interval. Repetition was chosen as one way to reduce amplitude to show that the reduction in the size of the cortical response and retinal responses is similar. The retinal response not having spontaneous potentials can be reduced to the vanishing point, whereas the cortical response can not, for when the response becomes small it is lost in the perpetual cortical activity whose waves are quite like the response itself. This is an experiment to show that the cortex responds to as high repetition frequencies as the retina.

Fig. 4. A comparison between cortical and retinal records when a two-stage flash is used as a stimulus. The first part (45$^{\sigma}$) was only 0.004 as bright as the second (25$^{\sigma}$). The retinal diagram shows also a response to a lengthened dim portion. Both the first and second responses in the retinal record are diphasic as evidenced by the increase in the negativity over the original wave to a homogeneous flash drawn in the solid line. Note that the cortical response is abrupt enough to avoid the overlapping shown in the retinal picture.

The curves also show that the cortical response dwindles in a manner similar to the retinal one. However, the former cannot be identified when it becomes small, on account of the spontaneous and also secondary response waves which mask it. The observer has therefore to be content with using the cortical response when it is large enough to stand out from the spontaneous activity and under conditions in which the secondary effects of two responses do not greatly overlap.

3. *Consecutive on responses.* A second stimulus may be given without the complication of intermediate *off* effect of the first by arranging the flash so that the first part of it is dim and the latter part bright with an abrupt transition between the two. A situation of this kind will be called one supplying two consecutive *on* stimuli. With such an arrangement a single response resembling the response to a homogeneous flash occurs in both the retinal and cortical records if the dim part of the flash is short. But by the time the dim segment is increased to 28–30$^\sigma$ (the bright 25$^\sigma$ and flash interval of 900$^\sigma$), a second peak will have definitely begun to appear on the descending limb of the positive phase of the retinogram as in figure 4. The original flash 55$^\sigma$ totally dim had elicited a wave beginning at 28$^\sigma$ with its peak at 61$^\sigma$. The new hump has a latency of about the same order as the first one. If the dim segment is made still longer, the hump appears later, as expected. In both cases the swing of the negative phase below the baseline is accentuated, showing the essential diphasic nature of the retinal response (*b* wave).

Dim segments of 8 and 16$^\sigma$ do not produce a second peak in the cortical record, but as in the retinal response, cause an increased latency over that of a flash, all of which was of the intensity of the bright segment. The delay for both the above values was equal (8$^\sigma$). When the dim segment was made 45$^\sigma$ long, two definite responses appeared, without the overlapping of the positive phases which characterized the retinal record. This is shown in figure 4.

4. *Short dark intervals.* Short dark intervals interrupting an otherwise continuous bright light produce no effects in the retinal record. Granit and Riddell (1934) found only the faintest trace to a 22$^\sigma$ dark interval with the illumination they used. A 100$^\sigma$ dark interval presented once every 1800$^\sigma$ produces only a slight response with our highest brightness. With a 35$^\sigma$ interval no response is recorded, while with a 52$^\sigma$ interval there is a trace.

On the other hand, the cortical record shows a definite response to both the beginning and ending of the interval; in other words, both a definite *off* and an *on* response. Under optimal conditions these appear with an interval as short as 12 sigmas.

Under none of the conditions used did our flashes or dark intervals produce a definite retinal *off* response. For conditions favorable to the retinal *off* response appearing in the retinogram, see Granit (1933).

5. *A comparison of the cortical on and off responses.* The fact alone that an *off* response appears at the cortex would suggest its existence as a neural phenomenon in the eye. But Hartline's finding of an *off* discharge in optic nerve fibres already had proved that the *off* response exists as a neural process there. If it fails to occur in the retinal record when it appears under similar conditions in the cortical record, it must be because

it is masked in the algebraic summation of it with other simultaneous processes and not for other reasons.

On account of the fact that the *off* response can be better isolated at the cortex, a comparison of cortical *off* and *on* responses follows.

The implicit time of the *off* response, like that of the *on*, can be varied by changing stimulus intensity. Figure 5A is a typical curve and is similar to that for the *on* response (Bartley, 1934). The response also varies considerably in size. These findings are contrary to the report of Wang (1934) in which it was stated that the size and latency of the *off* response does not vary, but in his case it is probable that the amount of

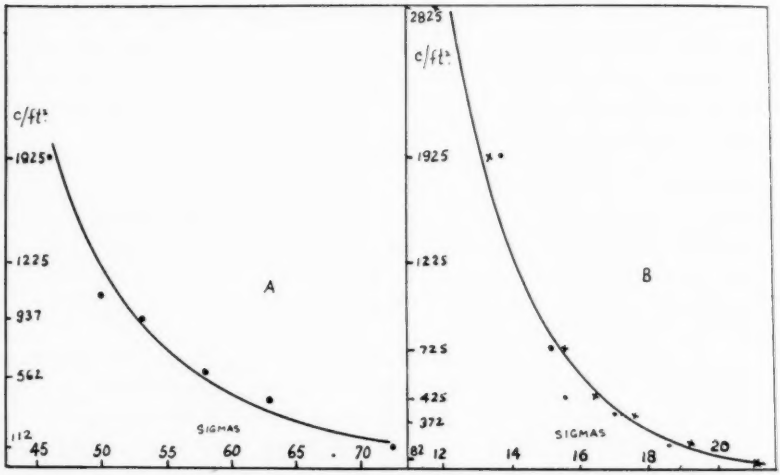

Fig. 5. A. The relation between flash intensity and the implicit time of the *off* response. Implicit time is the latency as measured to the peak of the response potential. This points out a similarity between the *off* and *on* responses.

B. The relation between intensity of flash necessary to produce an *off* response and the duration of the dark interval.

light used represented a level too high to produce a measurable response variation. The relation of the observable threshold of response to intensity and duration (fig. 5B) is a typical quantity curve, as was the threshold curve for the *on* effect, previously shown (Bartley, 1934).

The implicit times of the *on* and *off* responses of the cortex are not similar and may be compared by several kinds of experiments. If a constant illumination is interrupted by a dark interval, both an *off* and an *on* response will occur, in this case the *off* response, of course, preceding the *on*. The implicit times of both can be measured. If, on the other hand, a dark period is interrupted by a flash of light, both an *on* and an

off response may be observed, depending on the length of the flash. Comparing the implicit time of the *off* response for the beginning of the dark interval, and the *on* response for the flash, one will find that for the same intensity of illumination, the latency of the *off* response is much shorter. Or, comparing the implicit times of the *on* and *off* responses for a single flash or for a single dark interval, of similar duration, one will also find the implicit time of the *off* response shorter.

If light flashes are very short, no identifiable *off* response will follow the *on*.

On the other hand, if a dark interval is used, and the *off* response thus made to precede the *on*, the latter will follow the *off* very closely. Intervals as short as 12^{σ} may produce responses of nearly equal size to both the *off* and *on* (Bartley, 1936). The fact that the two types of responses can follow each other so closely suggests a more or less separate pathway clear to the cortex maintained by the differently stimulated fibres, for when similar pathways are activated a second time, no response will be elicited so soon after the first. That is, paired electric shocks to the optic nerve can not be spaced so closely and produce a response.

The *off* response, like the *on*, may exhibit at least a second wave following the first at a considerable distance. For example, when a dark interval is the stimulus, such as in the case above, three potentials can be observed to clearly rise out of the spontaneous activity. In a typical case, their peaks appeared at about 67, 115 and 162^{σ} respectively as measured from the *beginning* of the dark interval. Assuming the first to be the *off* response, its implicit time was 67^{σ}, and the third to be the *on* response, its implicit time was about 122^{σ}. In order to assign the second potential to the proper stimulus, the dark interval was lengthened by adding about 25^{σ} on to the beginning. In this case the three peaks appeared at 39, 86 138^{σ} as measured from the beginning of the original dark interval, which was the beginning of the line on the oscillograph. By adding 25^{σ} on to the first two values, they will approximately match the original readings. They will be 64, 111 and 163, as compared to 67, 115 and 162. From this, the second of the three responses belongs to the *off* effect and with an implicit time of about 110 to 115^{σ}.

Or, in another case in which the beginning of the oscillograph line was synchronized with the *end* of the dark interval, it being about 25^{σ} long, responses appeared at 48, 110 and 175^{σ}. Counting the first as an *off* response, 25^{σ} had to be added, making the implicit time 73^{σ}. The second wave was the *on* response (see below) with an implicit time of 110^{σ}. In this experiment the third wave is the second *on* response. It is possible that the second *off* response did appear in the record also, but we were unable to definitely identify it amidst the spontaneous variations occurring in the record or to dissociate it from the wave complex in the region

of 110$^{\sigma}$ where it would likely have appeared. When 47$^{\sigma}$ were added to the beginning of the dark interval, waves at 67 and 110–115$^{\sigma}$ definitely appeared. The total dark period was now 72$^{\sigma}$ long, bringing the first *off* response at or before the beginning of the oscillograph line. The first wave on the line (67$^{\sigma}$) was taken as the second *off* response, and the second as the first *on* response. The second *on* response also appeared part of the time. On other occasions when no succeeding *on* stimulation was introduced to complicate the situation, a wave has been observed to follow the *off* response at an interval of about 154$^{\sigma}$ or more. The *on* respones under corresponding conditions also included a second wave in the neighborhood of 162$^{\sigma}$. It is probable that this wave in the two cases compares with the corresponding one to electric stimulation of the optic nerve, in which it has been measured as early as 164$^{\sigma}$ (Bartley and Bishop, 1933).

The appearance in both cases of a second wave reveals another similarity in the general mechanism of the *off* and *on* responses. And its location identifies it with the second wave of the response to electrical stimulation.

Discussion. The purpose of presenting the foregoing facts was to make clear the use of cortical implicit time as an index of retinal behavior in ways similar to the present use of the retinal *b* wave. Inasmuch as the cortical recording site is quite remote from the retina, it is natural that doubt should arise at first regarding the use of any measure of cortical latency to study retinal behavior. It happens, however, that the synaptic junction (lateral geniculate body) acts in a way to make this possible. The delay at this point is not great and facilitation there condenses the burst of impulses arriving first into a relatively short and abrupt wave appearing as the first part of the cortical response.

The experiments show that the cortical record follows the retinal in its essential changes due to varying stimulus conditions, especially if the peaks of the two waves are used as points of reference. The further useful feature of the cortical response is that it represents an analysis of the activity of two of the kinds of discharge patterns, referable to the two groups of fibres found by Hartline, namely, those fibres discharging with a burst at the onset of the light flash and those discharing at its cessation.

Furthermore, the *off* response when it occurs has all the essential features of an *on* response and must be regarded as occupying a different set of elements, which, however, behave in the same way as those responsible for the *on* response. This is at variance with the conception that the *off* response is simply a restimulation due to a release of PII from the inhibitory effect of PIII. Creed and Granit (1933) definitely state that PII alone is associated with the discharge of impulses along the optic nerve and the negative PIII is some process which tends to actively inhibit it. In their conception, the *off* discharge represents a post-

inhibitory rebound of the *b* process, by implication activating the same fibres as initially activated by this process. If to Hartline's finding of separate fibres activated at *off* and *on*, is added the present finding that the interval between an *on* and an *off* response of the cortex can be less than that possible between two *on* responses, the mechanisms of the *on* and *off* effects appear to be separate throughout the pathway, and no inhibiting process is demanded to account for them. A cortical response may appear, even when the envelope of the retinal record fails to show a separate elevation to account for it.

The change in form of the retinal record with the duration of illumination raises a further question as to the significance of its configuration. The diphasic character of the *b* response to a short stimulus suggests that the elements responding to cause the *b* elevation give individually diphasic responses. The apparent monophasicity of the response to a prolonged illumination must be a function of the particular summation envelope that prolonged stimulation of such (repetitive?) elements results in. The *off* response when it occurs might then also be interpreted as the index of similar activity in different elements, its second phase, if any, being observed in the general falling off of potential following the cessation of stimulation.

SUMMARY

Certain conditions were selected in which to compare the *b* wave of the retinal record with the first wave of the cortical response. The following are the results.

1. The cortical response is more abrupt than the retinal and the possibility of its revealing certain things that may be masked in the retinal record is shown.

2. The two responses parallel each other rather closely in their latency changes with changing conditions.

3. They parallel each other rather closely in their reductions in size when the flash rate is increased, but the cortical response can not be used when small.

4. The cortical response shows a definite *off* response when no such wave is evident in the retinal record.

5. The behavior of the cortical *off* response is at variance with the concept that the *off* response is a post-inhibitory rebound, by implication involving the same optic nerve elements as the *on* response.

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RESPIRATORY REACTIONS UPON VERTICAL MOVEMENTS[1]

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The vegetative effects of labyrinthine reflexes upon vertical movements seem of importance in seasickness and airsickness. Little is known, however, about such reflexes. Graham Brown (1904) found in frogs that up and down movements induce first an increase of the intrapulmonary pressure. In the second part of the reaction movements of the lungs were missed, and only slight oscillations of the intrapulmonary pressure were noticed, that were probably due to slight contractions of the wall of the lungs. Pozerski (1921) was unable to produce symptoms of seasickness with his rocking apparatus in mammals such as guinea pigs, rabbits, even if they were exposed to the movements of this machine for 6 hours. Yet in 30 per cent of the dogs vomiting, polypnea, pollakisuria appeared. Sjöberg (1931) could provoke the typical symptoms of seasickness (increased salivation, polypnea, diarrhea, polyuria, psychic agitation, later apathy, vomiting) with all dogs under study when the animals were exposed to rapid up and down movements in a crane. The question, however, arises how far these symptoms are reflex phenomena due to labyrinthine or other reflexes upon the vegetative system, and how far they are secondary to the emotional excitation. Such an analysis seems particularly desirable in regard to the respiratory changes in mammals, since the anxiety and unrest of the seasick animals can produce by themselves respiratory reactions. The present investigation deals with an analysis of the respiratory reactions in vertical movements.

METHOD. Pitching movements were imitated by a rocking board upon which the animals (cats and dogs) were fastened. (Distance of the rotation axis from the end of the board, 1½ m., vertical distance of the highest and of the lowest position, 97 cm.) The rocking movements were produced by a motor with a variable frequency of 18 or 36 up and down movements per minute. Purely vertical movements were produced by connecting the end of the rocking board through a pulley with a horizontal animal holder that slid up and down between two vertical bars. Special care was taken to avoid accessory movements of the animal board, particularly

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concussion, since such movements may by themselves produce respiratory or vasomotor reactions. One has further to prevent the effect of inertia. In the turning points, the animal's body tries to continue its movement, while the movement of the board is already reversed. This plays a rôle particularly, when the downward movement is changed into an upward movement, since the impact of the board against the thorax may induce reflexly respiratory reactions. Such reactions are particularly noticeable if the animal is in prone position; they were avoided by fastening the animal in supine position and fixing the head in its holder in such a way that the upper thoracic part of the back did not touch the board. It was established that the noise of the motor did not produce any respiratory reaction. The respiration was recorded by a Harvard pneumograph fastened around the chest or by connecting a Y shaped cannula inserted into the trachea with the recording tambour. The up and down movements were recorded by a signal magnet connected with an electrical switch that was automatically closed at the lowest position of the animal.

Results and comment. Both types of movements, the purely vertical as well as the pitching movements, produced an increase of amplitude and frequency of respiration in unanesthetized animals. This effect was observed in dogs and cats already in the beginning of the oscillations; it was even often most pronounced at the start, while vomiting appeared in dogs much later, if at all, and did not appear in cats. Sjöberg also observed in his dogs the vomiting only 11 to 30 minutes after the beginning of the vertical movements. Yet a single movement or 2 or 3 up and downward movements may be able to elicit a respiratory reaction; the downward movement proved to be somewhat more effective than the upward movement. When the animals were continuously exposed to these vertical oscillations, some showed a decrease of the reaction after a few minutes, in others the change in respiration was sustained for ten and more minutes. Not infrequently the amplitude of the respiratory movements increased and decreased periodically in slow waves of $\frac{1}{4}$ to $1\frac{1}{2}$ minute duration.

The increase of the amplitude affected inspiration and expiration, the former effect appearing on the records of the thoracic respiration, the latter reaction appearing particularly on the graphs taken from the trachea. After the vertical movements were stopped, the amplitude of the respiratory movements was often diminished as compared to the respiration of the resting animal before the elevator movements had started. In other cases, however, an afterreaction outlasting the stimulation could be observed. Under superficial anesthesia (ether, dial-ether, morphine-ether anesthesia), the increase in the rate of respiration became less pronounced, while the increase in amplitude was still distinct. Under deep anesthesia the change in frequency as well as in amplitude subsided.

Effect of removal of higher centers. Typical respiratory reactions, such as

initial increase of the respiration, waves of increase and decrease in amplitude, were still observed after extirpation of both cerebral hemispheres (fig. 1, B). Extirpation of the hypothalamus in addition to removal of the cerebral hemispheres with the optic thalamus proper also did not abolish this reaction. When a transverse section behind the midbrain was performed, the respiratory reactions were markedly diminished, but not completely abolished. The conclusion seems justified that the respiratory changes induced by vertical movements are only partly due to psychic excitation, that, besides this factor, brain stem reflexes play an important part. In a few decerebrate animals an inhibition of the respiration (diminution of the amplitude) was noticed at the beginning of the movements. Such a reaction was particularly observed, when the respiratory center was in a poor condition, e.g., due to loss of large quantities of blood (fig. 1, D).

Receptors. Absence of *optical* impulses does not prevent the above mentioned respiratory reactions, as shown after severance of the brain stem behind the entrance of the optic tract into the external geniculate body. This, however, does not exclude the possibility that impulses from the retina may participate in the production of these reactions. The influence of vertical movements was, therefore, studied before and after the eyelids were closed by a tape. This had no definite effect upon the respiratory reactions on otherwise normal animals. If, however, more effective receptors were already eliminated (doublesided labyrinthectomy, transverse section of the cord), the remaining respiratory reactions on vertical movements were sometimes slightly diminished, when the eyelids were closed. Optical impulses seem, therefore, to have but a slight contributory influence in the stimulating effect of vertical movements upon respiration.

Severance of the *trigeminal* nerves in decerebrate animals did not change the respiratory reactions. The result was the same in animals with intact labyrinths as in animals with paralyzed labyrinths.

Doublesided *labyrinthectomy*, bilateral severance of the eighth nerve, or paralysis of the labyrinths produced by injection of formalin through the round window markedly diminished the respiratory reaction. The elimination of the labyrinthine receptors, however, was not quite sufficient to abolish it. Extirpation of the labyrinths was, therefore, combined with transverse section of the *spinal cord*. In some experiments the operation on the spinal cord preceded, in others it succeeded the labyrinthectomy. It was, of course, necessary to perform the severance of the cord below the origin of the phrenic nerve. Usually the level of the last cervical segment or between this segment and the first thoracic segment was chosen. Such an operation by itself already reduced the respiratory reaction upon the vertical movements, while control stimuli, e.g., from the skin of the face

proved that the respiratory centers and tracts still reacted. After additional bilateral labyrinthectomy, the respiratory reactions on vertical

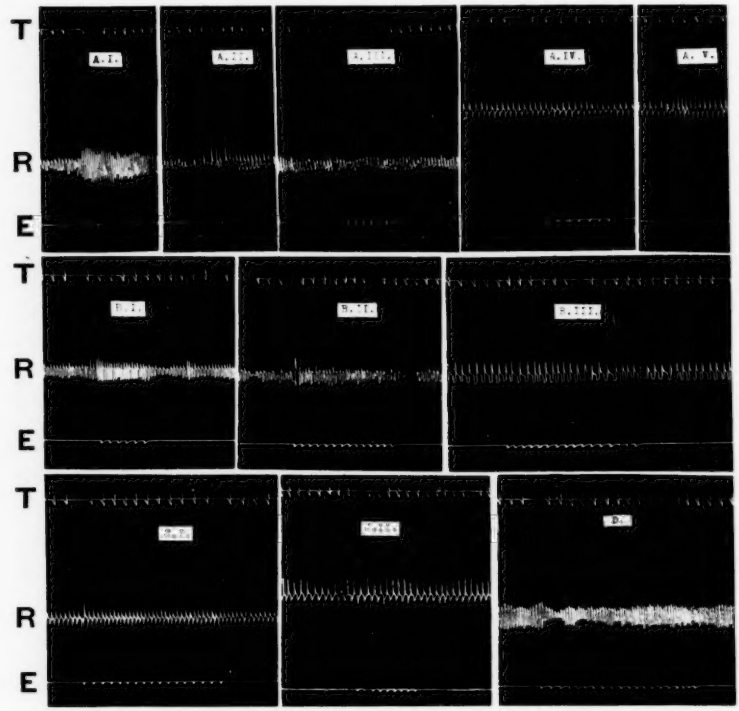

Fig. 1. Decerebrate and decorticate cats. A. Decerebrate cat. A.I. Respiratory reaction upon vertical movements. A.II. After transverse section between cervical and thoracic segments. A.III. Immediately. A.IV. Twenty minutes after formalin injection into both labyrinths. A.V. Control: reaction upon pinching the left foreleg. B.I. After extirpation of the cerebral hemispheres and the optic thalami; hypothalamus preserved. B.II. Hypothalamus extirpated. B.III. After transverse section behind the midbrain. C. Transverse section of the brainstem cranially to the midbrain. C.I. Respiratory reaction after transverse section of the first thoracic segment and severance of the cervical plexus. C.II. Respiration after formalin-paralysis of both labyrinths. D. Severe hemorrhage after decerebration. Inhibitory reaction.

R = respiration from trachea (expiration upwards). E = elevator movements (purely vertical movements). T = 5 second intervals.

movements were in some decerebrate animals no longer demonstrable (fig. 1, A). In some experiments traces of the reaction were still noticed. When the transverse section of the cord between the cervical and the

thoracic segments was in a decerebrate cat combined with severance of the cervical plexus (except the phrenic nerve), then subsequent labyrinthectomy completely abolished the respiratory reaction upon vertical movements (fig. 1, C). Control experiments showed that the respiratory center still reacted upon stimuli, e.g., from the area of the trigeminal nerve. As to the spinal tracts that carry the stimulating impulses to the respiratory center, it was found that bilateral labyrinthectomy plus severance of the posterior column and of the dorsal spinocerebellar tracts in the first cervical segment diminished but did not completely abolish the respiratory reaction upon vertical movements in decerebrate cats. This indicated that ascending systems in the lateral columns of the cord sending collaterals to the respiratory center (ventral spinocerebellar, or spinothalamic tract) may also coöperate in the reaction.

The inference may be drawn from all these experiments that impulses from the labyrinth, from the retina and finally impulses reaching the central nervous system with the spinal nerves (probably kinesthetic impulses from the skin and muscles) participate in producing the respiratory reactions upon vertical movements. Among these three groups, the impulses from the labyrinth and from the areas of the spinal nerves play (at least in the animals under study) a much more important part than the retinal impulses.

Relation to other vegetative labyrinth reflexes. It seemed necessary to analyze whether the labyrinthine impulses act primarily upon the respiratory center, or whether the reaction of this center is only secondary to labyrinthine reflexes upon other parts of the vegetative nervous system. Since stimulation of the labyrinth by rotation induces reflexly a fall of blood pressure (Spiegel and Démétriades, 1922), it seemed of interest to ascertain whether vertical movements have a similar effect upon the vascular system, and whether such a vasomotor reflex plays a part in the genesis of the above-mentioned respiratory changes. The blood pressure was recorded from the carotid or from the femoral artery by a mercury manometer. Since the manometer did not participate in the vertical movements of the animal, the changes of the animal's position in the vertical plane in relation to the manometer induced by themselves differences in the position of the recording lever. Various control experiments were, therefore, necessary. On dead animals, the carotid artery was connected with the manometer in exactly the same way as in the actual experiments. In controls on living animals the blood pressure was recorded with the animal held in the highest as well as in the lowest position of the elevator. A comparison of these control experiments with the records of blood pressure during the elevator movements gave no evidence that such an extent of vertical movements as produced in these experiments induces reflexly a change of blood pressure similar to the depressor effect of rotation. This

is particularly valid in pure rectilinear movements in the vertical plane. In the pitching movements sometimes a fall of blood pressure was noticed, particularly in the beginning of the movements or after they were stopped. Simultaneous records of the respiration showed that these changes were associated with single deep inspirations. In other cases, however, the blood pressure rose in the beginning of the pitching movements, while the change in respiration was the same as usual. The inference may be drawn that the changes in respiration brought about by vertical movements are not the consequence of reflexes upon the vasomotor center and of impaired blood supply of the respiratory center. They are also not caused by reflexes upon the digestive tract, as they appear also in animals that show no disturbances in this system. This indicates that impulses induced by vertical movements act primarily upon the respiratory centers.

SUMMARY

1. The influence of rhythmical rectilinear vertical movements and of pitching movements upon respiration was studied in dogs and cats.

2. Both types of movements induced an increase in frequency and amplitude of respiration. The effect is often most marked in the beginning of the movements, sometimes slow waves of increase and decrease in amplitude appear. The effect may outlast the stimulation, or may be followed by a period of inhibition (diminished amplitude of the respiratory movements).

3. In superficial anesthesia first the increase in the rate of respiration is affected, while the increase in amplitude during the vertical movements is still present. Deep anesthesia completely abolishes the reaction.

4. After extirpation of the cerebral hemispheres, the thalami, and the hypothalamus, the various types of the reaction could still be observed; if the reactivity of the respiratory center was depressed, sometimes an inhibitory reaction appeared. It is concluded that the respiratory reactions on vertical movements are at least partly due to brain stem reflexes.

5. An analysis of the importance of the various receptors in this reaction by extirpation experiments showed that mainly impulses from the labyrinth and from the area of the spinal nerves participate in producing the respiratory reactions upon vertical movements, while retinal impulses play only an accessory part.

6. These respiratory reactions appear independently of reactions of the vasomotor and digestive apparatus.

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TEMPORAL SUMMATION IN PERIPHERAL NERVE FIBERS[1]

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The phenomenon of temporal summation of subliminal afferent volleys to produce a reflex via the axial nervous system has long been known, but we have been unable to find any description of a comparable phenomenon in an axis cylinder. That temporal summation of impulses may occur in depressed peripheral nerve became apparent during the course of our observations on single axons, and this paper records our investigation of the phenomenon.

METHODS. The apparatus employed in general has been that used in the study of single axon spike potentials (Blair and Erlanger, 1933). The RCA 907 cathode ray tube now in use has proved sufficiently actinic to permit of the photographing of single sweeps across the face of the tube. The photographs, taken on 35 mm. motion picture film, are much sharper than the larger contact prints heretofore made by applying a film to the face of the tube. In all experiments on single axons the isolated phalangeal preparation of *Rana pipiens* has been employed. Usually by stimulation of one of the trunks of the sciatic plexus a preparation may be secured in which only one fiber conducts from the stimulated point to the lead electrodes on the distal attenuated portion of the nerve. The activity of this single fiber is observed by recording its amplified action potential. The rate of rotation of the interrupter synchronizing sweep and stimulation has been approximately 25 per minute. The first of the paired stimuli employed (designated S_1) has been the break shock from a Porter induction coil, the second (S_2), the discharge of a condenser through a grid-controlled, gaseous discharge tube (RCA 885) activated by the break of the primary current acting through the usual variable delay circuit. The RC product of the condenser discharge circuit has usually been of the order of 7.5×10^{-5}. Nonpolarizable Hg-calomel electrodes have been used exclusively. The potential difference between adjacent electrodes has rarely exceeded 3 millivolts.

RESULTS. *Initial observation of summation through accidental depression.* It frequently happens during observations on single axons, if and as con-

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ductivity fails, that a stage is reached during which a single adequate stimulus at a remote point never elicits a response at the lead, whereas a shock delivered after the first, and at the same point, results in a spike that is conducted the whole way, but only, of course, when it follows the first. If S_1 is weakened, at a critical level the response to S_2 disappears, but returns when S_1 is increased to its original strength. If the two shocks are approximated the response at the lead disappears at a critical interval; when separated beyond this value, it reappears. Two adequate shocks consistently result in a response at the lead when a single shock, even when increased many times above threshold, never causes a spike to appear. Apparently S_1 is producing a response which does not reach the lead, but modifies the nerve so that a second response, initiated in proper relation to the first, is conducted through.

This conclusion has been substantiated by a chance observation on an axon which gave a normal spike at both lead electrodes, but was obviously depressed in the interelectrode region: the response to S_1 was conducted to the electrode proximal with respect to the stimulus, but was blocked in the interelectrode region so that the spike was monophasic. Under these conditions the response to S_2, following S_1 within a wide time range, was invariably conducted through the depressed region to the other electrode, as indicated by the diphasicity of the response. When S_1 was adjusted to threshold so that the fiber was stimulated in about half the trials (see Blair and Erlanger, 1933), and S_2 kept at a superthreshold level, whenever there was a response to S_1, the response to S_2 would reach the distal electrode and produce a diphasic spike; whenever S_1 failed to stimulate, the response to S_2 would be blocked before reaching the distal electrode, and the spike would be monophasic and identical with that produced by S_1. Since the experimental conditions remained constant in this experiment, the shock escapes can be eliminated as a possible factor, and the passage of the second impulse can be referred to a change in the depressed region resulting from the preceding impulse which reached, but did not pass through, the interelectrode region.

Summation through experimental depression. Having established the fact that there are chance conditions under which a single impulse will not be conducted the full length of a fiber while the second of two impulses will, if initiated within an adequate interval, the attempt was made to find a means of producing a similar condition experimentally in normal nerve. First the effect was determined of passing a constant current through a localized region between the stimulus and the lead. By careful adjustment of the intensity of the current the phenomenon was elicitable in every experiment of this type.

The question then arises, does the summation develop in the catelectrotonic or in the anelectrotonic region? To determine this, the nerve, to

describe a typical experiment, is continuously polarized through the lead electrodes by means of a circuit that has been described elsewhere (fig. 1, Erlanger and Blair, 1934). The lead and polarizing circuits are identical, so that it is possible to follow the development of anodal polarization at one electrode, and of cathodal polarization at the other. Since block occurs at the anode with weaker currents than at the cathode, the lead electrode proximal with respect to the stimulus is made anode. When the current is increased the amplitude of the spike recorded from the anode increases and the positive phase of the spike recorded from the cathode decreases in amplitude until a critical current strength is reached, when, due to block at the anode, there is a sudden decrease in the height of the negative phase of the spike and the spike becomes monophasic (see Erlanger and Blair, 1934). In figure 1 the spike, R_2 of *A1* (produced by S_2 alone), has been reduced in height and made monophasic by this procedure. When, under these conditions, the fiber is stimulated earlier by S_1, as in *A2*, the early response, R_1, likewise is monophasic and low, but the second response, R_2, is higher and diphasic; the latter now is being conducted to the distal lead. The interval between the shock artifact, S_2, and the spike, R_2, represents the conduction time. The artifact of S_1 in all cases is off of the record. Occasionally, as in *A3*, both responses may be blocked at the node nearest the lead. That the same phenomenon appears at other adequate separations of S_1 and S_2 is shown in the comparable series, *B*.

When the polarizing current is further increased the spike amplitude falls, again abruptly (as in *C1*), due to the blocking of another segment. But, as before, when the nerve is conditioned by R_1 the response, R_2, is increased (as in *C2*) by the entrance of a previously blocked segment (compare *B1* and *C2*). Occasionally, however, when two segments are thus blocked conduction is completely restored by the conditioning response; an instance is seen in *C3* (compare *C3* with *B2*). The latter observation may be interpreted as indicating that the response of a segment affects not only the next segment beyond the block, but also the second segment removed. As this phenomenon has been observed in but one preparation, for the present the possibility of an extension of this effect further than one segment must be left open, though the evidence seems to favor it.

Some determinations have been made of the order of magnitude of anodal polarization required to demonstrate temporal summation in peripheral nerve. Briefly, the voltage is of the order of 1 to 2 rheobases, and in some cases, with a small nerve, temporal summation has occurred with a current of less than 0.1 microampere. The usual current ranges between 0.7 and 2 milliamperes per square centimeter cross-section of nerve. The impression has been gained that if the rheobase is low the nerve will show temporal summation at or near rheobase; that if the rheobase is high voltages up to

FIG. 1

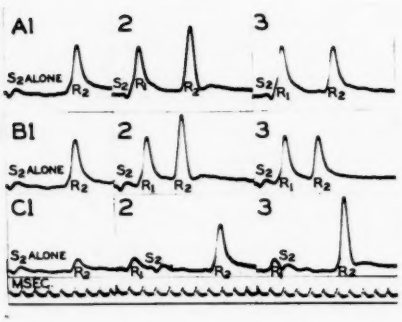

FIG. 2

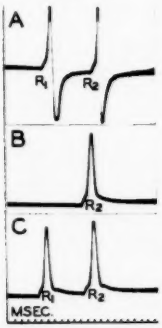

FIG. 3

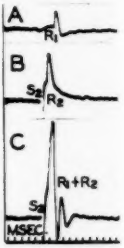

FIG. 4

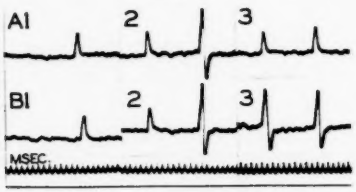

FIG. 5

Fig. 1. Temporal summation in a fiber blocked by a current through the lead electrodes, anode proximal to the stimulus. The records are from the same single axon. 5/21/36. All figures are reproduced unretouched and in photographed size.

A. 1. The normal diphasic spike converted to a lower monophasic spike, R_2, by anodal block. The spike is conducted to, but not past, the proximal lead.

2. Reëstablishment of the higher, diphasic response, R_2, by an antecedent, blocked response, R_1.

3. An infrequent record showing block of both spikes, R_1 and R_2.

B. 1, 2 and 3. As above, except for a shorter interval between the two spikes.

C. 1. Polarization increased so as to block an additional segment.

2. Reëstablishment of this segment response by temporal summation.

3. An infrequent result showing reëstablishment of conduction to the distal electrode by temporal summation through the two blocked segments.

Fig. 2. Temporal summation with multifiber spikes. 5/20/36.

A. Response (R_1 and R_2) of unpolarized nerve to two stimuli.

B. The spike, R_2, made monophasic and lower by anodal block at the proximal lead.

C. The height of response, R_2, increased by the antecedent response R_1 (Cf. R_2 of B).

Fig. 3. Records (5/14/36) showing summation of the effect on a blocked locus of an impinging response.

A. The nerve is stimulated by S_1 at a remote point, and the multifiber spike (about 12 fibers) reduced by anodal block.

B. Nerve stimulated by S_2 at the point of block so that only a few fibers respond.

C. Result of stimulation by S_2 at the time the spike from S_1 reaches the blocked locus.

2 rheobases may be required. If currents of this magnitude do not obtain physiologically, they probably are produced in the usual experimental preparation by demarcation potentials from sectioned fibers, and could very well be the cause of some, at least, of the incidental blocks that are encountered from time to time.

When the proximal electrode is made the cathode and the current increased while observing paired responses separated by about 15 msec., a stage is reached during which the first response of the pair is conducted through, while the second varies between full (cathodally polarized) height and a consistent, lower height. Apparently this stepdown in the height of the second spike is due to the failure of a previously conducting segment to respond. If the interval between the stimuli is reduced the amplitude of the second response falls off in steps in a manner entirely comparable to the quantal blocking resulting from increasing polarization (Erlanger and Blair, 1934). Manifestly in catelectrotonic nerve an effect develops that is the converse of temporal summation: a response makes the fiber less able to conduct a succeeding response.

The mechanism of temporal summation. Using anodal polarization as a means of producing a block which may be overcome by temporal summation of impulses, an effort was made to discover the underlying mechanism of this phenomenon. First, we endeavored to ascertain the shape of the curve of the temporal summation. Since summation is tested by a second response which, because of its all-or-none character, is not subject to independent experimental modification, the usual technique cannot be employed of expressing the effect of a conditioning stimulus in terms of the threshold of a testing stimulus. Only two points on the curve can be determined, namely, the minimum and the maximum separation of the two stimuli which will cause the second response to traverse the depressed locus. An effort was made to plot the curve indirectly by determining

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Fig. 4. Temporal summation in a single axon depressed at the proximal lead by calcium. 5/29/36.

A. 1. Calcium block converts a diphasic response to a lower, monophasic response.

2. An antecedent unconducted response now results in propagation of a second response to the distal lead.

3. Record when both responses are blocked.

B. 1 and 2. After block of both responses by calcium; temporal summation reëstablished by a current of 0.02μA with cathode at the poisoned region.

3. Calcium action antagonized by a further increase in cathodal polarization so that both responses are conducted.

Fig. 5. Temporal summation of several impulses. 5/19/36.

A. Effect on a repetitive burst of slight anodal polarization at the proximal lead.

B. Increasing the polarization increases the number of responses required to overcome the block. In this record 7 spikes reach the lead before conduction across it occurs. Time intervals, 5 msec.

these two values for a range of polarization strengths, but the technique did not yield quantitative information because the nerve continues to change under polarization and the rate of change appears to vary with the polarizing current.

It seemed possible that in a large nerve a number of fibers might exhibit temporal summation at a given polarization level, and, if so, that the curve might be determined approximately by the number of fibers, indicated by the amplitude of the multifiber spike, showing summation at several separations of the stimuli. With this in mind, to describe a given case, the two shocks, both maximal for alpha (see fig. 2*A*), were separated by about 7 msec. As polarization through the leads was increased with anode proximal the amplitude of the response from S_2 at first increased, due (a) to the anodal increase in the height of the contributing spikes and (b) to the cathodal reduction of the diphasic artifact, and then decreased as the individual fibers were blocked. When the amplitude has thus been reduced to about half of the maximum height (fig. 2*B*), the effect of an antecedent response was determined on the height of R_2. There was (see *C*) an increase amounting approximately to 6 per cent. No significantly greater values were found at other separations. The smallness of the increase is a reflection of the narrowness of the range of polarization which will produce a state showing temporal summation; in a multifiber response relatively few fibers are in this state at any given polarization strength. Obviously, the experimental range is not sufficiently wide to permit of the determination of a satisfactory curve of temporal summation.

Correlation of a large number of observations on single fibers in different preparations subjected to varying degrees of polarization indicates that the summation effect attains an early maximum and then declines slowly. Frequently the second response will be conducted through the blocked area as soon as the untreated nerve will conduct. We have seen summation with a stimulation interval of only 0.8 msec., when the second spike, though delayed through conduction in relatively refractory nerve, occurs so early on the negative after-potential that it appears to terminate the first spike. It is possible, however, for the second impulse to arrive at the lead too early to be propagated; this has occasionally been seen, but in no case has the appearance of summation been delayed longer than 3 msec. Apparently the curve reaches a maximum at from 2 to 4 msec. The decline of the effect, as indicated by wide changes in maximum summation time with slight changes in polarizing current, is very gradual. That the decline is slow is indicated also by the fact that there is a wide time range between conduction of all second responses and failure of all, signifying that summation declines by an amount equal to the range of the spontaneous change in the excitability of the axon over a relatively long period (Blair

and Erlanger, 1936 a and b). Very frequently the period of summation has lasted in excess of 100 msec.

Since a second response is conducted through a depressed region when the first is not, the phenomenon might be attributable to 1, an increase in the stimulating ability of the last responding segment, or 2, an increase in the excitability of the segment which failed to conduct the first response.

1. If the action potential is not actually responsible for restimulation it, at least, seems justifiable to assume that it is proportional to the stimulating power of any responding segment. Granting this, it follows that if the stimulating power of the last conducting segment is increased by a preceding response, this should be manifested as a supernormality of spike amplitude. To ascertain whether or not spike height is increased a state may be produced, by suitable polarization strength, in which the first response always is blocked, while the second is conducted in a vast majority of the trials. If temporal summation is referable to supernormality of spike height, then in the infrequent instances of failure of the second response to be conducted, the spikes which just fail of conduction should be higher than the first spike which never causes a response of the blocked segments. Figure 1, *A3* was secured under these conditions. R_1 never was conducted through, whereas R_2 failed infrequently. Certainly R_2 is no higher than R_1. *B3* was secured under the same conditions except for a shorter interval between stimuli. The second potential, even when the spike is added to the after-potential of the first, is definitely lower. Temporal summation, therefore, cannot be referred to an increase in the voltage of the response of the segment adjacent to the block due to a previous response, and inferentially temporal summation cannot be referred to an increase in the stimulus acting on the depressed locus.

2. Since the site of temporal summation apparently is not the last unblocked segment, the summation must be attributed to a change in the excitability of the blocked segment and should be demonstrable as a change in its electrical threshold. This deduction could be tested if a locus on a fiber could be blocked and the threshold determined there before and after the arrival of a spike which reached, but did not traverse, the locus. The experiment, however, is not feasible with the present preparation because the whole operation would have to be confined to a single trunk of the sciatic plexus in order to limit the response at the lead to a single fiber, and the trunk is not long enough for the purpose. Therefore the attack has been made by stimulating the whole sciatic nerve, instead of one of the trunks, and securing a record of the summed spikes of about twelve fibers, instead of but one. By polarizing through a pair of electrodes, with anode proximal to the lead and about 5 mm. from it, the spike is reduced by blocking all except two or three of the fibers responding to a shock (S_1) applied

at the central end of the nerve (fig. 3*A*). The proximal amplifier lead on the distal end of the nerve is then connected to record from the blocking electrode and the conduction time from S_1 to the depressed locus is determined. The amplifier is then reconnected to the original lead electrode and a shock (S_2) is adjusted to stimulate two or three fibers at the blocked locus (record *B*). When the two shocks, S_1 and S_2, are simultaneous, the response to the latter is not modified, showing that there is no interaction of shocks. When S_2 is applied to the polarized region, say, 30 msec. after the nerve has been stimulated by S_1, the presence of the first shock does not alter the response to S_2. But if the interval between shocks is then reduced, at about 20 msec. the addition of the first shock causes additional fibers to respond to the second. Still more fibers respond to the second shock as it is brought closer to the first, the increase produced by the latter apparently reaching a maximum when the separation is equal to the previously determined conduction time from the upper stimulating electrodes to the depressed locus. In record *C*, the two shocks having this separation produce a much higher spike ($R_1 + R_2$) than the sum of the two responses of *A* and *B* when the shocks are delivered separately, indicating the stimulation of additional fibers. When the second shock is delivered after an interval that is less than the conduction time, its spike precedes that from the earlier, more distant, stimulus and is not modified by it. The experiment demonstrates that a fiber response which is not conducted through a depressed segment, nevertheless may lower the threshold of the segment to electrical stimulation, and, inferentially, to a second propagated spike. In addition it can be taken to demonstrate that the curve of summation of shock and spike at a depressed locus is at a maximum approximately at the time of arrival of the spike, and then decreases at a very slow rate. It may not be superfluous to add that the total period of lowered threshold greatly outlasts the duration of the spike.

If the lowering of the threshold of a depressed segment produced by a spike impinging on it is maximal approximately coincidental with its arrival, one would expect the same relation to hold when dealing with temporally separated spikes. But, as mentioned above, the greatest temporal summation appears to occur with an interval between two spikes of from 2 to 4 msec. This difference apparently is to be referred to the fact that the refractory phase of the last conducting segment lasts some 2 to 4 msec., whereas the increased excitability of the blocked segment seems to fall very slowly; and an early, and consequently low, second spike in the last conducting segment may not stimulate beyond although the excitability of the blocked segment then is highest, whereas a later spike of normal amplitude will stimulate the still, though less, hyperexcitable segment.

Temporal summation through other than electrical blocks. All of the

observations on temporal summation that have been considered up to this point could be simply accounted for as being due to the effect of the negative action potential in opposing the polarizing current producing the block, the reduction of the induced depression then permitting a second response to be conducted. In order to ascertain whether this is a necessary combination other blocking agents, not involving external currents, have been employed. Ether, a commonly used blocking agent, produced a block with the same general characteristics as that elicited by cathodal polarization. From the point of view of behavior of the blocked region etherized and catelectrotonic nerve are indistinguishable.

Calcium, chosen because of the similarity of its action to anodal polarization, produced a block which showed temporal summation identical with that demonstrable by anodal polarization. In these experiments 1 per cent calcium chloride was jetted into the proximal lead electrode (see Erlanger and Blair, 1934). As diffusion raised the calcium concentration in the nerve the nerve response varied in a manner paralleling the variation produced by increasing anodal polarization, although, as indicated by the absence of current in the usual polarizing circuit, the electrodes remained essentially isoelectric. Figure 4, *A* is a series of records, comparable with those of figure 1, *A* and *B*, but showing temporal summation through a calcium block. A concentration of calcium below that required to block elevates the rheobase and reduces the amount of current necessary for block, apparently adding its effect to that of the anodal blocking current; and it subtracts from the cathodal rheobasic current. After the concentration of calcium had increased to the point of blocking both of the paired responses, the nerve was polarized cathodally at the depressed locus by 0.02 microampere and, as shown in *B1* and *2* of figure 4, temporal summation again could be demonstrated. That calcium acts in a manner analogous to anodal polarization is further shown by the fact that if cathodal polarization is increased, a calcium depression can be overcome so that both the first and the second responses will be conducted (see *B3*).

Figure 4 shows in *A3* the only case of supernormality of spike height observed in the whole series of experiments with polarization and with calcium. Anodal polarization and calcium both produce blocks which can be overcome by temporal summation without supernormality of spike height, but the present case indicates that under certain conditions with calcium, and possibly with other agents, increased response of the unblocked segment may play a part.

Temporal summation with repeated conditioning responses. Since the decreased threshold resulting from a spike impinging on a blocked segment far outlasts the refractory phase, it should be possible to sum the effect of several unconducted spikes to finally produce propagation through a block. An opportunity to study this phenomenon was presented by a fresh

preparation in which a single shock caused a repetitive response of the single axon conducting to the lead. By adjusting the strength of a blocking current with anode at the lead the picture could be progressively changed from the usual result of a single conditioning response blocked at the lead causing conduction of subsequent responses (fig. 5*A*) to one in which the effect of seven conditioning responses summed to eventually produce conduction (5*B*). A similar summation of the effect of blocked spikes is shown in a figure previously published (fig. 3, Erlanger and Blair, 1936). There the first few monophasic responses serve to condition a depressed locus between the leads so that the spike is permitted to pass, as indicated by the subsequent diphasic responses. The occasional monophasic responses occurring in that figure after a diphasic series has been started is referable, presumably, to a long refractory phase in the depressed locus.

Discussion. The evidence that the reactivity of a blocking segment can be raised by a blocked impulse seems to be indubitable; it is possible, even, that two adjoining segments can be thus affected. Taken at their face value, these observations imply that the last responding segment exerts an influence that radiates beyond the segment's limits a distance spanned by one or possibly two segments, roughly 1 or 2 mm. Electricity seems to be the only adequate agency that could radiate that distance in the time available. Our observations, therefore, signify that propagation in nerve is accomplished by a mechanism that is in part, at least, electrical. It does not seem possible to attribute the summation to a mechanism involving the liberation of neurohumors.

Temporal summation commonly is supposed to be an attribute of synaptic junctions in the nervous system, and synaptic junctions are characterized primarily by the discontinuity of the connection between the contiguous elements. This supposition is supported by the fact that an artificial discontinuity or block along the course of a fiber provides the means of demonstrating temporal summation.

It was stated above that we have not succeeded in finding in the literature any evidence for temporal summation in nerve fibers. There has been described, however, a phenomenon which simulates it. Bethe and others (see Schaefer and Schmitz, 1933, for the literature), using a nerve-muscle preparation, have found that local compression of the nerve at an intermediate point increases the height of the contractions of the muscle elicited by stimulation of the nerve with induction shocks. Now, one might conceivably maintain that in this experiment the compression serves as a second stimulus which sums temporally with the effect produced by the shock. Schaefer and Schmitz show, however, that the multifiber spike of a nerve actually is lowered by passing it through a compressed locus. Of significance in this connection is the evidence they present indicating that

injured fibers may continue to discharge for a time after conducting an impulse. Presumably the fibers under these circumstances respond with a brief repetitive burst (see Erlanger and Blair, 1936) and the increased height of the contraction in experiments with a nerve-muscle preparation, therefore, is to be attributed, not to temporal summation in the nerve, but to summation of contractions in the muscle,—to the conversion of a twitch into a brief tetanus.

SUMMARY

1. A state in a single axis cylinder such that a single impulse will not be conducted, but impulses succeeding within about 100 msec. will, can be produced by anodal polarization and by calcium poisoning, and also occurs fortuitously in failing nerve.

2. This temporal summation is referred to a change in the excitability of the blocked segment induced by the first response, which, though blocked, lowers the threshold beyond so that the next response, which may be lower, and typically is no higher, than the first, reaches the threshold of, and is conducted through, the depressed area.

3. Cathodal polarization and ether produce a state in nerve such that the second of two responses will not be conducted when the first is. By approximating the two stimuli under these circumstances it is possible to decrease the second spike in steps in a manner entirely comparable to the serial blocking of segments induced by increasing the intensity of depressing agents.

We are indebted to Dr. Hubert Peugnet for assistance in the designing of our present electron oscillograph plant. Doctor Peugnet collaborated with us during the initial observations of this investigation.

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CONTROL OF URINE FORMATION IN THE FROG BY THE RENAL CIRCULATION

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Do changes in the blood's circulation within the kidneys account for changes in the rates of urine formation (water excretion)? Ludwig (1844) suggested that such was the case. The opportunity to investigate the question for the frog arose because the circulation of blood through glomeruli could often be seen to change when water excretion stopped (Adolph, 1934; 1935a). Further observations showed that when the glomeruli were not visibly affected, other circulatory modifications could be identified.

Previous studies of glomerular circulation in amphibia were made, beginning with the work of Richards and Schmidt (1924) and Hill and McQueen (1921). In their studies, however, the rates of urine formation were not measured, so that the circulatory factors could not be correlated with actual performance in the kidneys. The notion has become prevalent, nevertheless, that glomerular blood flow is an index to urine formation. Some attempts to correlate urine formation with blood flow were made by Tamura et al. (1927), but urine formation was not measured over short intervals of time, foreign fluids were introduced into the circulation, and decisive factors were not employed to modify rates of urine formation.

The data of Richards and Walker (1935) and others affirm that in the frog fluid is separated from the blood while it is in glomerular capillaries. In the courses of the tubules, water and other constituents escape from the lumina. Hence the chief site of the control of volume output might be either in Malpighian bodies or in tubules, or equally in both.

METHODS. Each frog (*Rana pipiens*) was operated upon by crushing the brain, opening the abdomen, and exposing one kidney for observation. In the chief experiments the ureter leading from this kidney was cannulated, and while urine flowed into the cannula the position of the meniscus was read at one-minute intervals. In other experiments blood pressures were measured by connecting one cannulated aorta to a mercury manometer. In the kidney, selected glomeruli were observed by transmitted light, and presence or absence of blood flow in them was recorded. Heart rates were likewise observed. The entire frog was enclosed in a sealed

chamber and kept basally in an atmosphere of oxygen at atmospheric pressure, and usually at 20° to 22°C.

Changes in rate of urine production were induced by numerous agents. Agents that were in the gaseous state were administered by replacing the oxygen by another gas mixture. Agents that had to be applied intravenously or subcutaneously were injected from outside the chamber through very narrow rubber tubes. Agents applied to the skin were in solutions soaked up in cotton and placed on the skin by momentarily opening the chamber.

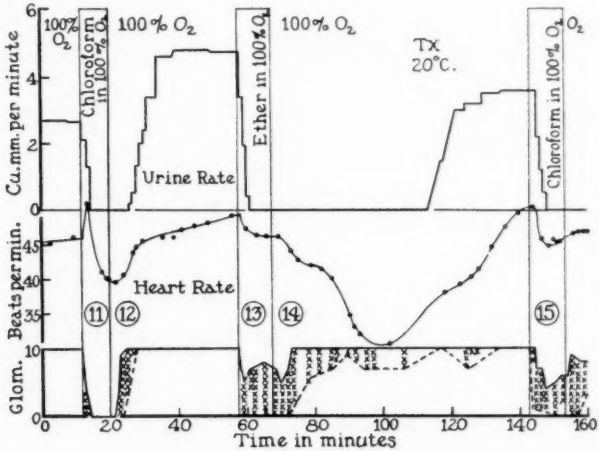

Fig. 1. Simultaneous rates of urine formation, pulse rate, and blood flow in glomeruli of frog *Tx*. The midbrain was crushed and the right ureter cannulated, and the animal was enclosed in a sealed chamber through which oxygen passed. The urine rates were measured by reading the meniscus in the ureter cannula at 1-minute intervals. In the right kidney ten glomeruli were watched; ordinates indicate the number of them through which blood flowed; the crosses indicate in which it flowed unusually slowly.

RESULTS. *Examples of the results.* In previous papers (1935a; 1935b; 1936) experiments were described in which simultaneously with cessation of urine formation all visible glomeruli ceased conducting blood. Both the circulatory changes and the changes in rate of water excretion occurred at the same moment, in response to administration of epinephrine or carbon dioxide, or to lack of oxygen. Upon the restoration of the basal conditions the circulation in the glomeruli was restored, and the urine formation resumed its previous control rate. Evidently the circulatory events were sufficient to account for the anuria, for it has been attested by many experiments that no urine forms when blood ceases to flow in all glomeruli.

Another type of experiment illustrating additional features is shown in figure 1; anesthetics were administered in the form of vapor while the atmosphere of oxygen was maintained. Urine rate, heart rate, and flow of blood in glomeruli changed almost instantaneously upon each administration of anesthetic. In the first administration (chloroform) it can be seen that the heart rate temporarily increased while the visible glomerular blood flow ceased entirely; hence it is evident that these two circulatory changes are not necessarily parallel. After the anesthetic was removed, the recovery of all three functions was prompt. At the second administration of anesthetic (ether) the blood did not stop flowing in all of the glomeruli, yet urine formation stopped completely. The recovery of glomerular activity was relatively prompt, but the heart rate now indicated grave

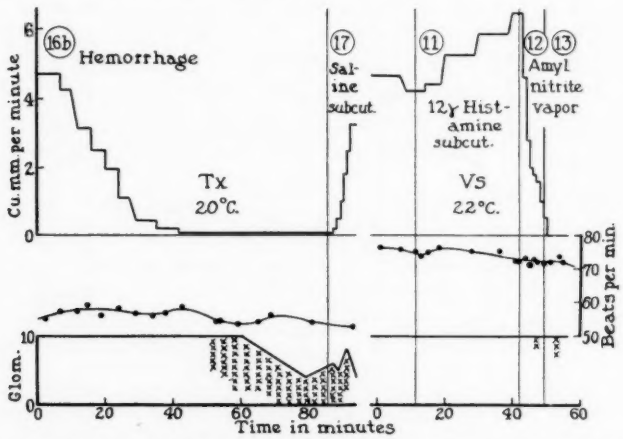

Fig. 2. Simultaneous rates represented in the same manner as in figure 1, in male frogs *Tx* and *Vs*, each weighing 26 grams and kept in an atmosphere of oxygen.

difficulty in the circulation; the systemic inadequacy coincided with the failure of urine formation for a period of 45 minutes. One notable feature was that the urine formation now became maximal before the heart rate reached its maximum. After the third administration of anesthetic the recovery of urine formation did not occur for a period of almost an hour, with prolonged but reversible slowing of the heart.

The experiments of figure 1 indicate the general facts which have been observed in numerous other experiments. These facts are: in order that urine formation shall proceed, blood must flow in at least some of the glomeruli *and* the general circulation must be in good condition. The converse does not hold, since even though the general circulation be excellent and some glomeruli be conducting blood, there may be no urine

formation. In other words, glomerular blood flow does not insure urine formation.

Other agents regularly produced oliguria or anuria without any specific effect upon the blood vessels leading to the glomeruli. An example is afforded by the hemorrhage experiment plotted in figure 2; the diminution of urine flow accompanied a reduction of glomerular blood flow and of heart rate. Recovery occurred very promptly when isotonic salt solution was injected into the lymph spaces. A similar change in the general circulation followed the administration of amyl nitrite vapor (fig. 2); the rate of heart beat also diminished rapidly in subsequent minutes.

Certain agents were found that produced oliguria without obviously modifying the rate of heart beat or the glomerular blood flow; examples

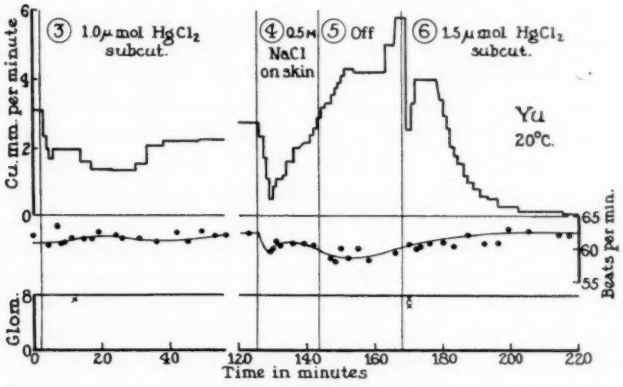

Fig. 3. Simultaneous rates represented in the same manner as in figure 1, in male frog *Yu* weighing 38 grams and kept in an atmosphere of oxygen. The two oliguric agents tested here produced no significant changes in heart rate and only momentarily in blood flow through glomeruli.

are shown in figure 3. Mercuric chloride, injected into lymph spaces in two different doses, reduced the urine output to 45 per cent of the basal rate and to zero; yet in neither case was any persisting reduction of the glomerular circulation manifested. A circulatory change in glomerular blood flow was regularly exhibited in the first 2 or 3 minutes after administration; this coincided with the initial fleeting oliguria at the time of 170 minutes (fig. 3). Occasionally renal arterioles constricted momentarily in response to almost any change of environment or administration of agent. Persisting circulatory changes occurred after mercuric chloride injection, for the amplitude of heart beat decreased progressively. In two of five tests, the rates of urine formation returned to normal spontaneously, and in *Yu* 6 the injection of a moderate amount of saline was followed

by temporary but marked urine production, without debris appearing in the urine. Another agent that gave little observable indication of influence upon glomerular blood flow or heart rate was a strong solution of sodium chloride placed on the skin (fig. 3). Recovery from this oliguria was spontaneous. For further analysis of the effects of these agents the measurement of arterial blood pressure was required.

Control experiments were found in the cases of several agents that failed to influence urine formation to significant extents. Ethyl urethane, when applied to the skin for a sufficient length of time to abolish reflexes, and histamine, injected subcutaneously, had no effect; and neither of them produced visible change in the circulation. Another control procedure, injection of isotonic sodium chloride solution, was without oliguric effect, as previously reported (Adolph, 1936).

Classification of oligurias. The degrees to which the renal *blood flows* were modified when urine formation was reduced by the various agents may be compared by the following procedure. The number of glomeruli being watched that maintained a flow of blood within their capillaries in the control period was recorded at brief intervals during the experimental period. The proportion of glomeruli that ceased to conduct blood was thus ascertained; further those glomeruli which conducted blood obviously more slowly than normal were credited with an activity of half the normal. Thus in the absence of total closure of any one glomerulus the activity was not evaluated at less than 50 per cent. It must be understood that the "glomerular activities" recorded have not the quantitative basis that the other changes possess. The *heart rates* were compared by taking the control period just previous to represent a rate of 100 per cent. The *urine rates* were compared in the same way, taking the previous control period as 100 per cent. All the control periods were characterized by an atmosphere of pure oxygen about the non-breathing frog.

The results of correlating these three quantities are given in table 1. Careful consideration of the data on circulatory conditions, particularly those of glomerular activity, leads to division of the experiments into five categories, and the agents tested are tabulated in an order that corresponds. In group *A*, the effect of the agents producing oliguria was to reduce the blood flow through the glomeruli and to stop it completely in a great many of them; yet the general circulation and the heart rate improved. In group *B*, the effect was likewise to reduce specifically the blood flow in glomeruli, but at the same time to embarrass the circulation in the whole body, with decrease in rate of heart beat. In group *C*, the reduction of blood flow in the renal glomeruli was only part of a depression of the systemic circulation, which was, however, entirely reversible. In group *D*, no circulatory change of magnitude sufficient to slow visibly the flow of blood through the glomeruli was noted; when the arterial blood pressure

was measured, significant changes were revealed. In group *E*, there was no significant oliguria and no visible effect upon the circulation. It is noteworthy that no agent was found that conversely produced a circulatory change without affecting urine formation.

Time relations. Further analysis of the correlation of circulatory and renal changes in response to various agents was made by comparing the times required for the onset of oliguria and of recovery from it. The

TABLE 1

Mean ultimate rates of urine formation, pulse rate, and approximate glomerular activity, as percentage of those in control periods, shown during exposures to various oliguric agents by pithed frogs at 19° to 26°C.

	AGENT	DOSE	NUMBER OF TESTS	NUMBER ANURIC	URINE RATE	HEART RATE	GLOMERULAR ACTIVITY
					per cent	per cent	per cent
A.	1. Epinephrine subcutaneously.	200–650 γ/kgm.	6	1	16	103	18
	Epinephrine by vein	26–310 γ/kgm.	8	1	27	104	24
	2. Infundin subcutaneously	2–92 units/kgm.	6	5	3	104	0
	Infundin by vein	0.8–1.6 units/kgm.	3	2	3	106	30
B.	3. Ethyl ether vapor		5	4	2	87	16
	4. Chloroform vapor		3	3	0	85	15
	5. Chloretone on skin	0.04 M	4	2	8	89	30
	Chloretone by vein	84 μ mols/kgm.	2	0	45	92	60
	6. Oxygen lack, *a*	0.2 per cent	11	11	0	72	0
C.	Oxygen lack, *b*	10–13 per cent	7	2	8	79	90
	Oxygen lack, *c*	21 per cent	5	0	25	94	100
	7. Carbon dioxide	29–31 per cent	9	7	2	95	70
	8. Amyl nitrite vapor		3	2	4	86	60
	9. Hemorrhage		2	1	10	91	32
D.	10. Na oxalate subcutaneously	350–600 μ mols/kgm.	3	1	30	96	83
	11. $HgCl_2$ subcutaneously	26–58 μ mols/kgm.	5	3	10	104	79
	12. Concentrated NaCl on skin	0.5 M	3	0	35	99	96
	13. Drying the skin		2	0	40	99	100
E.	14. Urethane on skin	1.1 M	3	0	76	98	100
	15. Histamine subcutaneously	63–630 γ/kgm.	3	0	128	102	100
	16. 0.1 M NaCl subcutaneously	5–22 cc./kgm.	4	0	156	104	100

results of such comparison are indicated in table 2. At the onset of oliguria, either the glomerular activity or the cardiac activity was obviously diminished simultaneously or within 1 minute of the first diminution of urine rate. This held for the individual experiments, as well as for the averages of many experiments.

In the recovery period the glomerular activity was restored rather promptly; the pulse rate either equally promptly, or, in the case of the

volatile anesthetics, with some delay. The urine rate often recovered much more slowly than the circulatory factors. Evidently the circulatory factors were prerequisite for urine formation, but adequacy of pulse rate and glomerular blood flow did not always insure urine formation. Some other changes which might recover more slowly than either of the measured circulatory changes observed appeared to be required in many instances.

Arterial blood pressures. In what circumstances are changes of heart

TABLE 2

Mean latent periods, in minutes, of earliest visible renal and circulatory changes simultaneously observed in the experiments listed in table 1

All rates decreased at onset and increased at recovery unless otherwise (†) noted. Some recovery times (*) are measured from the time of application of the agent instead of from its removal.

	AGENT	ONSET OF OLIGURIA			START OF RECOVERY		
		Urine	Heart	Glomeruli	Urine	Heart	Glomeruli
A.	1. Epinephrine subcutaneously	2	3†	2	10		5*
	Epinephrine by vein	1	2†	1	5		4*
	2. Infundin subcutaneously	2	2†	2	38		20*
	Infundin by vein	1	2†	2	16		10*
B.	3. Ethyl ether vapor	1	2	1	20	8	3
	4. Chloroform vapor	2	2	1	22	7	2
	5. Chloretone on skin	1	1	1	21		16
	Chloretone by vein	1	2	2	5		*
	6. Oxygen lack 0.2 per cent	2	3	2	7	1	2
C.	Oxygen lack 10–13 per cent	5	6	6	4	1	2
	Oxygen lack 21 per cent	6	7	8	6	2	10
	7. Carbon dioxide 29–31 per cent	3	4	7	10	3	4
	8. Amyl nitrite vapor	1	2	3	14	2	
	9. Hemorrhage	2	5	3	40		*
D.	10. Na oxalate subcutaneously	7	7	12	58		46*
	11. $HgCl_2$ subcutaneously	1	2†	1	30		*
	12. Concentrated NaCl on skin	2			6		*
	13. Drying the skin	6			2		
E.	14. Urethane on skin	1			7		*
	15. Histamine subcutaneously	6†					
	16. 0.1 M NaCl subcutaneously	1†	2		12		*

rate indicative of changes of blood pressure? Arterial pressures were measured in the aortic arches of frogs that were otherwise treated in the same ways as when rates of urine formation had been measured.

The results of the study of arterial pressures are shown in table 3. Here are included data of certain other investigators obtained under conditions that appeared to correspond closely to those prevailing in the present measurements of rate of urine formation. The most striking feature is

that the changes of arterial pressures correlate accurately with the changes of urine rates for agents 3 to 16 inclusive. The latent periods for the be-

TABLE 3

Mean ultimate aortic blood pressures and heart rates (as percentage of those in control periods), and latent periods, shown by operated frogs in brief exposures to various oliguric agents at 18° to 22°C.

* Frogs not operated; † Toads

	AGENT	DOSE		NUMBER OF TESTS	AORTIC PRESSURE	LATENCY OF ONSET	ULTIMATE EFFECT AT	HEART RATE
					per cent	*minutes*	*minutes*	*per cent*
A.	1. Epinephrine subcutaneously	5 γ		8	145 C		8	
	Epinephrine by vein	200–2500	γ/kgm.	7	133 K	1		
	2. Infundin subcutaneously	3.7	units/kgm.	2	106 N	1	8	103
	Infundin by vein							
B.	3. Ethyl ether vapor			3*	64 B			
	4. Chloroform vapor			2	79 N	1	5	84
	5. Chloretone on skin	0.04	M	2	82 N	4	11	93
	Chloretone by vein							
	6. Oxygen lack, *a*	0.2	per cent	8	64 A_1	2	8	75
C.	Oxygen lack, *b*	10–13	per cent	2	89 A_2	5	11	95
	Oxygen lack, *c*	21	per cent	4	82 N	5	21	96
	7. Carbon dioxide	29–31	per cent	3	75 N	5	17	84
	8. Amyl nitrite vapor							
	9. Hemorrhage			9†	72 H		3	95
D.	10. Na oxalate subcutaneously	250–400	μ mols/kgm.	3	81 N	5	15	101
	11. $HgCl_2$ subcutaneously	19–37	μ mols/kgm.	6	77 N	2	19	102
	12. Concentrated NaCl on skin	0.5	M	3	93 N	2	6	98
	13. Drying the skin							
E.	14. Urethane on skin	1.1	M	1	107 N	1	4	101
	15. Histamine subcutaneously			Few	100 C			
	16. 0.1 M NaCl subcutaneously	5–12	cc./kgm.	3	133 N	3	11	102

A_1 = Adolph, 1934; A_2 = Adolph, 1935b; B = Bieter and Scott, 1929; C = Cullis and Scarborough, 1932; H = Hofmeister, 1889; K = Kuno, 1914; N = new.

ginnings of pressure changes are in agreement with the latent periods for changes of heart rate shown in table 2.

The statement may be made that the rates of heart beat are indications

of the blood pressure changes produced by agents of groups *B*, *C*, and *E*, but are not so for group *D*. Evidently agents of group *D* diminish the systemic arterial pressures but do not affect the heart rates unfavorably.

Pressure responses to agents 10, 11, and 12 (group *D*) are portrayed in figure 4. Evidently marked decreases of pressure occurred in the first two, even though the heart rates remained constant or else increased slightly. To the third agent (0.5 M NaCl on the skin) only a temporary decrease was shown; but this corresponds exactly to the course of the oliguria occurring (fig. 3). As may be noted in figure 4, the arterial pressures, after decreasing, frequently failed to return to the original levels. Yet it is known that urine flow usually recovered promptly, and this may point to the occurrence of local adjustments in renal blood-vessels. Dry-

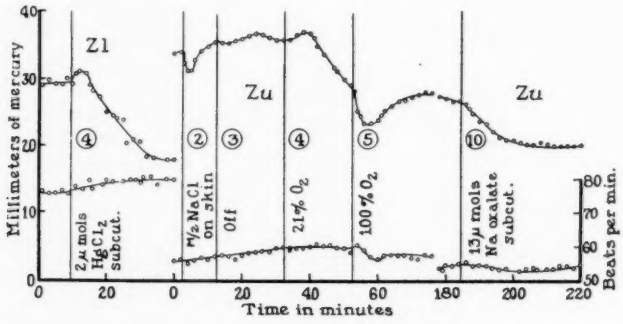

Fig. 4. Pressures in the left branch of the aorta, and simultaneous rates of heart beat. Frog *Zl* of 54 grams and frog *Zu* of 50 grams, kept in an atmosphere of oxygen. The character of the pressure changes may be compared with the urinary rate changes shown in *Yu* (fig. 3) in which similar doses of some of the same agents were administered.

ing the skin (agent 13) always involved some exposure of the frog to cooling by evaporation. Schulz (1906) found that a lowering of temperature from 18° to 8°C. decreased the arterial pressure to 79 per cent and the pulse rate to 30 per cent. It is concluded as the result of much study, however, that cooling is not the only factor involved in the oliguria thus produced; yet in any measurement of blood pressure this sequel of cooling will manifest itself while the skin is drying, masking other factors.

Measurements of aortic pressure (agent 6*c*) in the presence of room air (21 per cent oxygen) now showed distinct decreases (fig. 4) compared to those in the presence of 100 per cent oxygen. The fact was previously reported (Adolph, 1935b) that urine production of the non-breathing frog was slow in room air.

The times at which pressure effects occurred in *renal* vessels were also

measured. Changes in what may be referred to as "afferent arteriolar pressure" were ascertained by successively introducing fluid under pressure through the ureter into the urinary passages during 10 to 15 seconds, and noting at each imposition of pressure whether blood ceased to flow into the capillaries of certain glomeruli. It was ascertained that no further changes of flow occurred after 10 seconds of pressure. If the flow into one glomerulus stopped and not into another, this was taken to indicate the mean systolic pressure at the points of entrance of blood into the glomeruli. By this procedure it could be found whether pressures fell in the renal arterioles under the same circumstances as in the aorta.

An experiment is shown in figure 5. It was found that the local arteriolar pressure changed with the same latencies as the rate of heart beat.

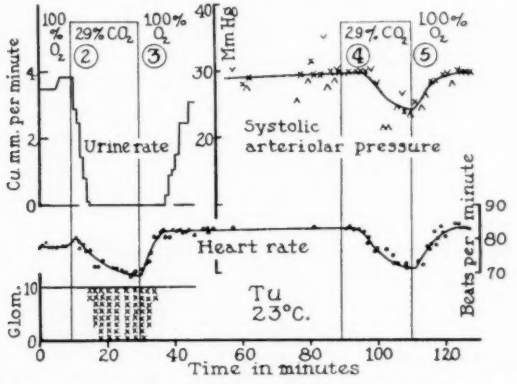

Fig. 5. Data for rate of urine formation, followed by data for systolic afferent arteriolar pressure as measured by imposing various pressures through the ureter. Two glomeruli were being watched; if flow in neither stopped in 10 to 15 seconds an upward arrow was recorded, if one stopped a cross, if both stopped a downward arrow. Male frog *Tu*.

The frog showed the same latency for oliguria as for decrease of heart rate upon administration of carbon dioxide, but when the carbon dioxide was removed the pressure *began* to recover more quickly than urine formation recovered.

The latent periods found in eight tests with 29 per cent carbon dioxide and 71 per cent oxygen were: renal arteriolar pressure began to decrease in 5 minutes; recovery (increase) began in 5 minutes. In seven tests with 11 to 21 per cent oxygen, the decrease of arteriolar pressure began in 4 minutes; recovery began in 2 minutes. These pressure changes were simultaneous with the other changes observed in the circulation (table 3), and therefore at the onset of oliguria were simultaneous with the first decrease of urine production (table 2).

No absolute significance can be attached to these local pressures. It may be mentioned that the mean value found in 29 control determinations upon six frogs that were in an atmosphere of 100 per cent oxygen was 28 mm. mercury. This may conceivably be compared with the average value of 23.5 mm. mercury obtained in afferent arterioles by Hayman (1927) using the method of introducing pressure into a single capsule through a microcannula, until the systolic inflow of blood ceased. The difference is not necessarily due to resistance to pressure transmission through the renal tissues, for the conditions of the animals were not identical (e.g., with respect to oxygen tension and to blood plethora) in Hayman's investigation and in the present work.

The amounts of change in afferent arteriolar pressure were usually greater than those in rate of heart beat. Thus in 11 per cent oxygen the pressure was 87 per cent of the control (3 tests); in 21 per cent oxygen was 78 per cent (4 tests); in 29 per cent carbon dioxide was 77 per cent (9 tests). They were nearly the same as those in mean aortic pressure (table 3). The tests of arteriolar pressure were limited to these agents of group *C*. It should not be concluded that the arteriolar pressure decreased in proportion to the general arterial pressure in response to all agents. In the instances of visible arteriolar constriction (agents 1 to 6*a*) such relationship is known not to hold.

Other features of the experiments in which pressure was imposed through the ureter may be mentioned here. In additional instances the cannula and tubing were not filled with saline to the leveling reservoir, but the meniscus was within the cannula. This allowed measurements of the rate of entrance of fluid into the kidney. The fluid entering did not all issue again when the pressure was released, and both the rate of entrance and the amount retained increased with the pressure imposed. But a larger proportion of the fluid was retained (up to 70 per cent of that entering) when high pressures were applied (30 to 35 mm. mercury). The rates of entrance and amounts of retention diminished with the duration of the pressure application, both in the living and in the dead animal. These and other facts illustrate first that the kidney is distensible and elastic, part of the distensibility being due to displacement of blood from the renal blood vessels; and second, that fluid escapes from the urinary passages into the renal tissues, and from the renal tissues into the blood. The amounts of fluid escaping into the kidney amounted to as much as 2 cc. in the course of 4 hours of repeated pressure application, while the kidney finally weighed only 100 mgm. The urine production meanwhile steadily increased with the blood plethora whenever periods for measuring its rate intervened.

Measurements of both aortic pressure and *glomerular* blood pressure were carried out by Hayman (1927) under normal conditions and in the presence of epinephrine and of intravenously injected saline. In some instances

diminution of these pressures after epinephrine injection was reported, but these were not in the majority. Obviously proper correlations may be found only by measuring the glomerular blood pressures under circumstances in which the effects on rates of urine output are known.

Summary of results. In response to each of the thirteen agents (groups *A* to *D*) that produced oliguria, there was some circulatory change. Three agents (group *E*) that did not interfere with urine formation left the circulation unaffected. No means was found of depressing the circulation without inhibiting urine excretion.

No response but oliguria occurred in any test of the thirteen agents reported to produce it, with two sorts of exceptions: epinephrine sometimes (9 out of 23 tests) brought on polyuria without the oliguric phase, as detailed in another paper (1936); and, in two tests not reported, polyuria was induced by an atmosphere of 21 per cent oxygen replacing 100 per cent oxygen.

COMMENT. To discover and study *all* the agents that can diminish the rate of urine formation in the frog is obviously an unmanageable task. But 16 agents represent a liberal sample; and since 13 out of 13 oliguric agents act primarily on the circulation, the expectation that the next oliguric agent tested would have no circulatory action should be very small, though it is incapable of statistical expression.

The question naturally arises whether several of the agents used to produce oliguria may not mediate their effects through some common factor. Thus, epinephrine might be liberated through the agencies of pituitary extracts, absence of oxygen, and other influences. In such a case several of the agents used would merely illustrate the effects of one final agent. There is no direct evidence that this is or is not the case.

Richards (1929) observed in frogs injected with mercuric chloride 24 hours previously that glomerular fluid was forming in certain capsules, while no urine was issuing from the ureter. His explanation was therefore that fluid was passing from the blood into the capsules at normal rates, but later completely escaped because of loss of selectivity of the tubular epithelium. The circulations have not been studied in frogs rendered anuric for 24 hours. The rates of urine formation by intact frogs have now been measured, however, and the occurrence of anuria for 24 hours or longer when large doses of mercuric chloride were injected subcutaneously was confirmed. Frogs whose anuria persisted for such a long period rarely recovered.

It is impossible to affirm that the agents used to induce oliguria have no action in the kidneys other than that upon the circulation. The general fact which emerges is that the effects upon the circulation are of such a nature, of such a magnitude, and occur at such a time as to account for the oliguria found.

The observations favor the supposition made by Ludwig (1844) that the pressure of the blood in the glomerular capillaries is effective in expelling water from the blood to form urine. Whatever may occur in the tubular structures of the kidney does not appear to interfere with the parallelism between glomerular condition and urine production, in spite of the fact that the absolute quantities of urine may still be modified by the tubular functions.

The question might arise why the conditions for polyuria were not studied along with those for oliguria. It is obvious, however, that the methods of observing the glomerular circulation were not adequate for detecting increases in it. Marked diminution of blood flow in glomeruli can be ascertained consistently, but augmentations would require quantitative measurement. Decreases of arterial pressure are almost certain to involve decreases of local blood pressure, but increases may easily be cut off by the interposed arterioles.

SUMMARY

1. Thirteen methods of producing decreases of urine formation in the frog were studied; all decreased the circulation of blood either locally or generally. Three additional agents that produced no oliguria produced also no circulatory changes.

2. Either one or both circulatory factors that were observed (the local glomerular blood supply or the general arterial pressure) correlated in degree with the rate of urine formation. In many instances the general circulatory condition could be judged by comparison of rates of heart beat; in others measurements of arterial blood pressure were required.

3. The latent periods of circulatory change were similar to those for the onset of oliguria; but in the recovery from oliguria the excretory rate might be restored some time after the circulation improved.

4. Since no oliguric agent was found that did not act on the circulatory system, it is suggested that urine formation was diminished in all the cases studied by decrease of mean blood pressure within glomerular blood vessels.

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